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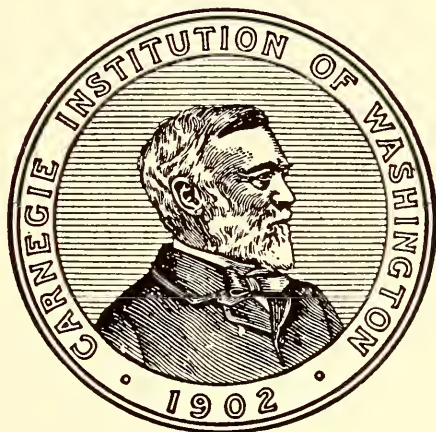


# CARNEGIE INSTITUTION OF WASHINGTON

YEAR BOOK No. 53

July 1, 1953—June 30, 1954

With Administrative Reports through December 10, 1954



*Copy for  
Director's  
Office*

CARNEGIE INSTITUTION OF WASHINGTON  
WASHINGTON, D. C.

1954



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## PRESIDENT AND TRUSTEES

### PRESIDENT

VANNEVAR BUSH

### BOARD OF TRUSTEES

ELIHU ROOT, JR., *Chairman*

BARKLIE HENRY, *Vice-Chairman*

ROBERT WOODS BLISS, *Secretary*

|                        |                    |                         |
|------------------------|--------------------|-------------------------|
| JAMES F. BELL          | BARKLIE HENRY      | WILLIAM I. MYERS        |
| ROBERT WOODS BLISS     | ERNEST O. LAWRENCE | HENNING W. PRENTIS, JR. |
| LINDSAY BRADFORD       | ALFRED L. LOOMIS   | DAVID ROCKEFELLER       |
| OMAR N. BRADLEY        | ROBERT A. LOVETT   | ELIHU ROOT, JR.         |
| W. CAMERON FORBES      | KEITH S. MCHUGH    | HENRY R. SHEPLEY        |
| WALTER S. GIFFORD      | ROSWELL MILLER     | CHARLES P. TAFT         |
| CRAWFORD H. GREENEWALT | HENRY S. MORGAN    | JUAN T. TRIPPE          |
| CARYL P. HASKINS       | SEELEY G. MUDD     | ROBERT E. WILSON        |

### *Executive Committee*

BARKLIE HENRY, *Chairman*

|                    |                  |                         |
|--------------------|------------------|-------------------------|
| ROBERT WOODS BLISS | CARYL P. HASKINS | HENNING W. PRENTIS, JR. |
| LINDSAY BRADFORD   | HENRY S. MORGAN  | ELIHU ROOT, JR.         |
| VANNEVAR BUSH      |                  | HENRY R. SHEPLEY        |

### *Finance Committee*

LINDSAY BRADFORD, *Chairman*  
ALFRED L. LOOMIS  
HENRY S. MORGAN  
HENNING W. PRENTIS, JR.  
ELIHU ROOT, JR.

### *Nominating Committee*

KEITH S. MCHUGH, *Chairman*  
CRAWFORD H. GREENEWALT  
ROBERT A. LOVETT  
ELIHU ROOT, JR.

### *Auditing Committee*

KEITH S. MCHUGH, *Chairman*  
BARKLIE HENRY  
DAVID ROCKEFELLER

### *Retirement Committee*

LINDSAY BRADFORD, *Chairman*  
BARKLIE HENRY  
HENRY S. MORGAN

---

### *Committee on Astronomy*

SEELEY G. MUDD, *Chairman*  
CRAWFORD H. GREENEWALT  
ROSWELL MILLER  
ELIHU ROOT, JR.

### *Committee on Biological Sciences*

CARYL P. HASKINS, *Chairman*  
ALFRED L. LOOMIS  
WILLIAM I. MYERS  
CHARLES P. TAFT

### *Committee on Terrestrial Sciences*

ERNEST O. LAWRENCE, *Chairman*  
BARKLIE HENRY  
DAVID ROCKEFELLER

### *Committee on Archaeology*

HENRY R. SHEPLEY, *Chairman*  
JAMES F. BELL  
ROBERT WOODS BLISS  
JUAN T. TRIPPE

## FORMER PRESIDENTS AND TRUSTEES

### PRESIDENTS

DANIEL COIT GILMAN, 1902-1904

ROBERT SIMPSON WOODWARD, 1904-1920

JOHN CAMPBELL MERRIAM, *President* 1921-1938; *President Emeritus* 1939-1945

### TRUSTEES

|                       |         |                       |         |
|-----------------------|---------|-----------------------|---------|
| ALEXANDER AGASSIZ     | 1904-05 | SETH LOW              | 1902-16 |
| GEORGE J. BALDWIN     | 1925-27 | WAYNE MACVEAGH        | 1902-07 |
| THOMAS BARBOUR        | 1934-46 | ANDREW W. MELLON      | 1924-37 |
| JOHN S. BILLINGS      | 1902-13 | DARIUS O. MILLS       | 1902-09 |
| ROBERT S. BROOKINGS   | 1910-29 | S. WEIR MITCHELL      | 1902-14 |
| JOHN L. CADWALADER    | 1903-14 | ANDREW J. MONTAGUE    | 1907-35 |
| WILLIAM W. CAMPBELL   | 1929-38 | WILLIAM W. MORROW     | 1902-29 |
| JOHN J. CARTY         | 1916-32 | WILLIAM CHURCH OSBORN | 1927-34 |
| WHITEFOORD R. COLE    | 1925-34 | JAMES PARMELEE        | 1917-31 |
| FREDERIC A. DELANO    | 1927-49 | WM. BARCLAY PARSONS   | 1907-32 |
| CLEVELAND H. DODGE    | 1903-23 | STEWART PATON         | 1916-42 |
| WILLIAM E. DODGE      | 1902-03 | GEORGE W. PEPPER      | 1914-19 |
| CHARLES P. FENNER     | 1914-24 | JOHN J. PERSHING      | 1930-43 |
| HOMER L. FERGUSON     | 1927-52 | HENRY S. PRITCHETT    | 1906-36 |
| SIMON FLEXNER         | 1910-14 | GORDON S. RENTSCHLER  | 1946-48 |
| JAMES FORRESTAL       | 1948-49 | ELIHU ROOT            | 1902-37 |
| WILLIAM N. FREW       | 1902-15 | JULIUS ROSENWALD      | 1929-31 |
| LYMAN J. GAGE         | 1902-12 | MARTIN A. RYERSON     | 1908-28 |
| CASS GILBERT          | 1924-34 | THEOBALD SMITH        | 1914-34 |
| FREDERICK H. GILLETT  | 1924-35 | JOHN C. SPOONER       | 1902-07 |
| DANIEL C. GILMAN      | 1902-08 | WILLIAM BENSON STOREY | 1924-39 |
| JOHN HAY              | 1902-05 | RICHARD P. STRONG     | 1934-48 |
| MYRON T. HERRICK      | 1915-29 | WILLIAM H. TAFT       | 1906-15 |
| ABRAM S. HEWITT       | 1902-03 | WILLIAM S. THAYER     | 1929-32 |
| HENRY L. HIGGINSON    | 1902-19 | JAMES W. WADSWORTH    | 1932-52 |
| ETHAN A. HITCHCOCK    | 1902-09 | CHARLES D. WALCOTT    | 1902-27 |
| HENRY HITCHCOCK       | 1902-02 | FREDERIC C. WALCOTT   | 1931-48 |
| HERBERT HOOVER        | 1920-49 | HENRY P. WALCOTT      | 1910-24 |
| WILLIAM WIRT HOWE     | 1903-09 | LEWIS H. WEED         | 1935-52 |
| CHARLES L. HUTCHINSON | 1902-04 | WILLIAM H. WELCH      | 1906-34 |
| WALTER A. JESSUP      | 1938-44 | ANDREW D. WHITE       | 1902-03 |
| FRANK B. JEWETT       | 1933-49 | EDWARD D. WHITE       | 1902-03 |
| SAMUEL P. LANGLEY     | 1904-06 | HENRY WHITE           | 1913-27 |
| CHARLES A. LINDBERGH  | 1934-39 | GEORGE W. WICKERSHAM  | 1909-36 |
| WILLIAM LINDSAY       | 1902-09 | ROBERT S. WOODWARD    | 1905-24 |
| HENRY CABOT LODGE     | 1914-24 | CARROLL D. WRIGHT     | 1902-08 |

Besides the names enumerated above, the following were ex-officio members of the Board of Trustees under the original charter, from the date of organization until April 28, 1904: the President of the United States, the President of the Senate, the Speaker of the House of Representatives, the Secretary of the Smithsonian Institution, the President of the National Academy of Sciences.

# STAFF OF INVESTIGATORS FOR THE YEAR 1954

## ASTRONOMY

### MOUNT WILSON AND PALOMAR OBSERVATORIES

*813 Santa Barbara Street, Pasadena 4, California*

Mount Wilson Observatory organized in 1904; George E. Hale, Director 1904–1923, Honorary Director 1923–1936; Walter S. Adams, Director 1924–1945. Unified operation with the Palomar Observatory of the California Institute of Technology began in 1948.

IRA S. BOWEN, *Director*  
WALTER BAADE  
HORACE W. BABCOCK  
WILLIAM A. BAUM  
ARMIN J. DEUTSCH  
JESSE L. GREENSTEIN  
MILTON L. HUMASON  
RUDOLPH L. MINKOWSKI

GUIDO MÜNCH  
SETH B. NICHOLSON  
DONALD E. OSTERBROCK  
EDISON PETTIT  
ROBERT S. RICHARDSON  
ALLAN R. SANDAGE  
OLIN C. WILSON  
FRITZ ZWICKY

## TERRESTRIAL SCIENCES

### GEOPHYSICAL LABORATORY

*2801 Upton Street, N.W., Washington 8, D. C.*

Organized in 1906, opened in 1907; Arthur L. Day, Director 1909–1936; Leason H. Adams, Acting Director 1936–1937, Director 1938–August 1, 1952; George W. Morey, Acting Director August 1, 1952–August 31, 1953.

PHILIP H. ABELSON, *Director*  
FRANCIS R. BOYD, JR.  
FELIX CHAYES  
GORDON L. DAVIS  
JOSEPH L. ENGLAND  
HANS P. EUGSTER  
JOSEPH W. GREIG

FRANK C. KRACEK  
GUNNAR KULLERUD  
WILLIAM S. MACKENZIE  
GEORGE W. MOREY  
J. FRANK SCHAIRER  
HATTEN S. YODER, JR.

### DEPARTMENT OF TERRESTRIAL MAGNETISM

*5241 Broad Branch Road, N.W., Washington 15, D. C.*

Organized in 1904; Louis A. Bauer, Director 1904–1929; John A. Fleming, Acting Director 1929–1934, Director 1935–1946.

MERLE A. TUVE, *Director*  
L. THOMAS ALDRICH  
ELLIS T. BOLTON  
ROY J. BRITTEN  
BERNARD F. BURKE  
DEAN B. COWIE  
JOHN W. FIROR  
SCOTT E. FORBUSH  
JOHN W. GRAHAM  
NORMAN P. HEYDENBURG  
\*ELLIS A. JOHNSON  
RICHARD B. ROBERTS  
HOWARD E. TATEL  
GEORGES M. TEMMER  
GEORGE R. TILTON  
ERNEST H. VESTINE  
HARRY W. WELLS  
GEORGE W. WETHERILL

### *Visiting Investigators*

S. J. AHMED  
WILLIAM R. DURYEE  
K. L. FRANKLIN  
‡PAUL W. GAST  
‡PEMBROKE J. HART  
‡H. LAWRENCE HELFER  
‡B. Y. MILLS  
‡LEIF OWREN  
FLOYD RECTOR  
IRENA Z. ROBERTS  
‡F. GRAHAM SMITH

\* On leave of absence.

‡ Term of appointment completed in 1954.

# CARNEGIE INSTITUTION OF WASHINGTON

## BIOLOGICAL SCIENCES

### DEPARTMENT OF PLANT BIOLOGY

*Stanford, California*

Desert Laboratory, opened in 1903, became headquarters of Department of Botanical Research in 1905; name changed to Laboratory for Plant Physiology in 1923; Daniel T. MacDougal, Director 1906–1927. Reorganized in 1928 as Division of Plant Biology, including Ecology; Herman A. Spoehr, Chairman 1927–1930 and 1931–1947, Chairman Emeritus 1947–1950. Name changed to Department of Plant Biology in 1951.

C. STACY FRENCH, *Director*

JENS C. CLAUSEN

\*PAUL GRUN

WILLIAM M. HIESEY

HAROLD W. MILNER

MALCOLM A. NOBS

JAMES H. C. SMITH

*Guest Investigator*

HEMMING I. VIRGIN

*Visiting Investigator*

ELIAS LANDOLT

### DEPARTMENT OF EMBRYOLOGY

*Wolfe and Madison Streets, Baltimore 5, Maryland*

Organized in 1914; Franklin P. Mall, Director 1914–1917; George L. Streeter, Director 1918–1940

GEORGE W. CORNER, *Director*

DAVID W. BISHOP

BENT G. BÖVING

ROBERT K. BURNS

ARPAD CSAPO

SAMUEL R. M. REYNOLDS

*Consultant*

GEORGE W. BARTELMEZ

*Research Associates*

CHESTER H. HEUSER

ELIZABETH M. RAMSEY

### DEPARTMENT OF GENETICS

*Cold Spring Harbor, Long Island, New York*

Station for Experimental Evolution opened in 1904; name changed to Department of Experimental Evolution in 1906; combined with Eugenics Record Office in 1921 to form Department of Genetics. Charles B. Davenport, Director 1904–1934; Albert F. Blakeslee, Director 1935–1941.

MILISLAV DEMEREC, *Director*

ALFRED D. HERSHEY

BERWIND P. KAUFMANN

BARBARA McCLINTOCK

MARGARET R. McDONALD

EVELYN M. WITKIN

*Special Investigators*

‡MAURICE H. BERNSTEIN

ELIZABETH BURGI

‡DOROTHY K. FRASER

ALAN GAREN

HELEN GAY

PHILIP E. HARTMAN

‡JEAN HEMMERLY

‡JUNE DIXON HUDIS

NORMAN E. MELECHEN

HERMANN MOSER

\* Resigned in 1954.

‡ Term of appointment completed in 1954.



## STAFF OF INVESTIGATORS FOR THE YEAR 1954

### ARCHAEOLOGY

#### DEPARTMENT OF ARCHAEOLOGY

*10 Frisbie Place, Cambridge 38, Massachusetts*

Department of Historical Research organized in 1903; Andrew C. McLaughlin, Director 1903-1905; J. Franklin Jameson, Director 1905-1928. In 1930 this Department was incorporated as a section of United States history in a new Division of Historical Research; Alfred V. Kidder, Chairman 1930-1950. Name changed to Department of Archaeology in 1951.

HARRY E. D. POLLOCK, *Director*

MARGARET W. HARRISON, *Editor*

EARL H. MORRIS

TATIANA PROSKOURIAKOFF

KARL RUPPERT

ANNA O. SHEPARD

EDWIN M. SHOOK

A. LEDYARD SMITH

ROBERT E. SMITH

GUSTAV STRÖMSVIK

J. ERIC S. THOMPSON

### RESEARCH ASSOCIATES

RALPH W. CHANEY (University of California), Paleobotany

LOUIS B. FLEXNER (University of Pennsylvania), Embryology

ARTHUR T. HERTIG (Boston Lying-in Hospital), Embryology

WILLARD F. LIBBY (United States Atomic Energy Commission), Chemistry

## OFFICE OF ADMINISTRATION

VANNEVAR BUSH, *President*

PAUL A. SCHERER, *Executive Officer*

SAMUEL CALLAWAY, *Assistant to the President*

AILENE J. BAUER, *Director of Publications*

DOROTHY R. SWIFT, *Editor*

EARLE B. BIESECKER, *Bursar*

J. STANLEY LINGEBACH, *Assistant Bursar*

JAMES W. BOISE, *Accountant*

JAMES F. SULLIVAN, *Assistant to the Bursar*

RICHARD F. F. NICHOLS, *Executive Assistant to the Finance Committee*

## OFFICERS OF RETIREMENT FUND

EARLE B. BIESECKER, *Secretary-Treasurer*

JAMES W. BOISE, *Assistant Treasurer*

## ORGANIZATION, PLAN, AND SCOPE

The Carnegie Institution of Washington was founded by Andrew Carnegie, January 28, 1902, when he gave to a board of trustees an endowment of registered bonds of the par value of ten million dollars. To this fund an addition of two million dollars was made by Mr. Carnegie on December 10, 1907, and a further addition of ten million dollars was made by him on January 19, 1911. Furthermore, the income of a reserve fund of about three million dollars, accumulated in accordance with the founder's specifications in 1911, is now available for general use, and in recent years a total of ten million dollars has been paid by the Carnegie Corporation of New York as increase to the Endowment Fund of the Institution. The Institution was originally organized under the laws of the District of Columbia and incorporated as the *Carnegie Institution*, articles of incorporation having been executed on January 4, 1902. The Institution was reincorporated, however, by an act of the Congress of the United States, approved April 28, 1904, under the title of *Carnegie Institution of Washington*. (See existing Articles of Incorporation on following pages.)

Organization under the new Articles of Incorporation was effected May 18, 1904, and the Institution was placed under the control of a board of twenty-four trustees, all of whom had been members of the original corporation. The trustees meet annually in December to consider the affairs of the Institution in general, the progress of work already undertaken, and the initiation of new projects, and to make the necessary appropriations for the ensuing year. During the intervals between the meetings of the trustees the affairs of the Institution are conducted by an Executive Committee chosen by and from the Board of Trustees and acting through the President of the Institution as chief executive officer.

The Articles of Incorporation of the Institution declare in general "that the objects of the corporation shall be to encourage, in the broadest and most liberal manner, investigation, research, and discovery, and the application of knowledge to the improvement of mankind."

The Institution is essentially an operating organization. It attempts to advance fundamental research in fields not normally covered by the activities of other agencies, and to concentrate its attention upon specific problems, with the idea of shifting attack from time to time to meet the more pressing needs of research as they develop with increase of knowledge. Some of these problems require the collaboration of several investigators, special equipment, and continuous effort. Many close relations exist among activities of the Institution, and a type of organization representing investigations in astronomy, in terrestrial sciences, in biological sciences, and in archaeology has been effected. Conference groups on various subjects have played a part in bringing new vision and new methods to bear upon many problems. Constant efforts are made to facilitate interpretation and application of results of research activities of the Institution.

## ARTICLES OF INCORPORATION

PUBLIC No. 260. An Act to incorporate the Carnegie Institution of Washington.

*Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,* That the persons following, being persons who are now trustees of the Carnegie Institution, namely, Alexander Agassiz, John S. Billings, John L. Cadwalader, Cleveland H. Dodge, William N. Frew, Lyman J. Gage, Daniel C. Gilman, John Hay, Henry L. Higginson, William Wirt Howe, Charles L. Hutchinson, Samuel P. Langley, William Lindsay, Seth Low, Wayne MacVeagh, Darius O. Mills, S. Weir Mitchell, William W. Morrow, Ethan A. Hitchcock, Elihu Root, John C. Spooner, Andrew D. White, Charles D. Walcott, Carroll D. Wright, their associates and successors, duly chosen, are hereby incorporated and declared to be a body corporate by the name of the Carnegie Institution of Washington and by that name shall be known and have perpetual succession, with the powers, limitations, and restrictions herein contained.

SEC. 2. That the objects of the corporation shall be to encourage, in the broadest and most liberal manner, investigation, research, and discovery, and the application of knowledge to the improvement of mankind; and in particular—

(a) To conduct, endow, and assist investigation in any department of science, literature, or art, and to this end to cooperate with governments, universities, colleges, technical schools, learned societies, and individuals.

(b) To appoint committees of experts to direct special lines of research.

(c) To publish and distribute documents.

(d) To conduct lectures, hold meetings, and acquire and maintain a library.

(e) To purchase such property, real or personal, and construct such building or buildings as may be necessary to carry on the work of the corporation.

(f) In general, to do and perform all things necessary to promote the objects of the institution, with full power, however, to the trustees hereinafter appointed and their successors from time to time to modify the conditions and regulations under which the work shall be carried on, so as to secure the application of the funds in the manner best adapted to the conditions of the time, provided that the objects of the corporation shall at all times be among the foregoing or kindred thereto.

SEC. 3. That the direction and management of the affairs of the corporation and the control and disposal of its property and funds shall be vested in a board of trustees, twenty-two in number, to be composed of the following individuals: Alexander Agassiz, John S. Billings, John L. Cadwalader, Cleveland H. Dodge, William N. Frew, Lyman J. Gage, Daniel C. Gilman, John Hay, Henry L. Higginson, William Wirt Howe, Charles L. Hutchinson, *Samuel P. Langley*, William Lindsay, Seth Low, Wayne MacVeagh, Darius O. Mills, S. Weir Mitchell, William W. Morrow, *Ethan A. Hitchcock*, Elihu Root, John C. Spooner, Andrew D. White, Charles D. Walcott, Carroll D. Wright, who shall constitute the first board of trustees. The board of trustees shall have power from time to time to increase its membership to not more than twenty-seven members. Vacancies occasioned by death, resignation, or otherwise shall be filled by the remaining trustees in such manner as the by-laws



## ARTICLES OF INCORPORATION

shall prescribe; and the persons so elected shall thereupon become trustees and also members of the said corporation. The principal place of business of the said corporation shall be the city of Washington, in the District of Columbia.

SEC. 4. That such board of trustees shall be entitled to take, hold, and administer the securities, funds, and property so transferred by said Andrew Carnegie to the trustees of the Carnegie Institution and such other funds or property as may at any time be given, devised, or bequeathed to them, or to such corporation, for the purposes of the trust; and with full power from time to time to adopt a common seal, to appoint such officers, members of the board of trustees or otherwise, and such employees as may be deemed necessary in carrying on the business of the corporation, at such salaries or with such remuneration as they may deem proper; and with full power to adopt by-laws from time to time and such rules or regulations as may be necessary to secure the safe and convenient transaction of the business of the corporation; and with full power and discretion to deal with and expend the income of the corporation in such manner as in their judgment will best promote the objects herein set forth and in general to have and use all powers and authority necessary to promote such objects and carry out the purposes of the donor. The said trustees shall have further power from time to time to hold as investments the securities hereinabove referred to so transferred by Andrew Carnegie, and any property which has been or may be transferred to them or such corporation by Andrew Carnegie or by any other person, persons, or corporation, and to invest any sums or amounts from time to time in such securities and such form and manner as are permitted to trustees or to charitable or literary corporations for investment, according to the laws of the States of New York, Pennsylvania, or Massachusetts, or in such securities as are authorized for investment by the said deed of trust so executed by Andrew Carnegie, or by any deed of gift or last will and testament to be hereafter made or executed.

SEC. 5. That the said corporation may take and hold any additional donations, grants, devises, or bequests which may be made in further support of the purposes of the said corporation, and may include in the expenses thereof the personal expenses which the trustees may incur in attending meetings or otherwise in carrying out the business of the trust, but the services of the trustees as such shall be gratuitous.

SEC. 6. That as soon as may be possible after the passage of this Act a meeting of the trustees hereinbefore named shall be called by Daniel C. Gilman, John S. Billings, Charles D. Walcott, S. Weir Mitchell, John Hay, Elihu Root, and Carroll D. Wright, or any four of them, at the city of Washington, in the District of Columbia, by notice served in person or by mail addressed to each trustee at his place of residence; and the said trustees, or a majority thereof, being assembled, shall organize and proceed to adopt by-laws, to elect officers and appoint committees, and generally to organize the said corporation; and said trustees herein named, on behalf of the corporation hereby incorporated, shall thereupon receive, take over, and enter into possession, custody, and management of all property, real or personal, of the corporation heretofore known as the Carnegie Institution, incorporated, as hereinbefore set forth under "An Act to establish a Code of Law for the District of Columbia, January fourth, nineteen hundred and two," and to all its rights, contracts, claims, and property of any kind or nature; and the several officers of such corporation, or

## CARNEGIE INSTITUTION OF WASHINGTON

any other person having charge of any of the securities, funds, real or personal, books, or property thereof, shall, on demand, deliver the same to the said trustees appointed by this Act or to the persons appointed by them to receive the same; and the trustees of the existing corporation and the trustees herein named shall and may take such other steps as shall be necessary to carry out the purposes of this Act.

SEC. 7. That the rights of the creditors of the said existing corporation known as the Carnegie Institution shall not in any manner be impaired by the passage of this Act, or the transfer of the property hereinbefore mentioned, nor shall any liability or obligation for the payment of any sums due or to become due, or any claim or demand, in any manner or for any cause existing against the said existing corporation, be released or impaired; but such corporation hereby incorporated is declared to succeed to the obligations and liabilities and to be held liable to pay and discharge all of the debts, liabilities, and contracts of the said corporation so existing to the same effect as if such new corporation had itself incurred the obligation or liability to pay such debt or damages, and no such action or proceeding before any court or tribunal shall be deemed to have abated or been discontinued by reason of the passage of this Act.

SEC. 8. That Congress may from time to time alter, repeal, or modify this Act of incorporation, but no contract or individual right made or acquired shall thereby be divested or impaired.

SEC. 9. That this Act shall take effect immediately.

*Approved, April 28, 1904*

## BY-LAWS OF THE INSTITUTION

Adopted December 13, 1904. Amended December 13, 1910, December 13, 1912, December 10, 1937, December 15, 1939, December 13, 1940, December 18, 1942, December 12, 1947, and December 10, 1954

### ARTICLE I

#### THE TRUSTEES

1. The Board of Trustees shall consist of twenty-four members, with power to increase its membership to not more than twenty-seven members. The Trustees shall hold office continuously and not for a stated term.
2. In case any Trustee shall fail to attend three successive annual meetings of the Board he shall thereupon cease to be a Trustee.
3. No Trustee shall receive any compensation for his services as such.
4. All vacancies in the Board of Trustees shall be filled by the Trustees by ballot at an annual meeting, but no person shall be declared elected unless he receives the votes of two-thirds of the Trustees present.

### ARTICLE II

#### OFFICERS OF THE BOARD

1. The officers of the Board shall be a Chairman of the Board, a Vice-Chairman, and a Secretary, who shall be elected by the Trustees, from the members of the Board, by ballot to serve for a term of three years. All vacancies shall be filled by the Board for the unexpired term; provided, however, that the Executive Committee shall have power to fill a vacancy in the office of Secretary to serve until the next meeting of the Board of Trustees.
2. The Chairman shall preside at all meetings and shall have the usual powers of a presiding officer.
3. The Vice-Chairman, in the absence or disability of the Chairman, shall perform the duties of the Chairman.
4. The Secretary shall issue notices of meetings of the Board, record its transactions, and conduct that part of the correspondence relating to the Board and to his duties.

### ARTICLE III

#### EXECUTIVE ADMINISTRATION

##### *The President*

1. There shall be a President who shall be elected by ballot by, and hold office during the pleasure of, the Board, who shall be the chief executive officer of the Institution. The President, subject to the control of the Board and the Executive Committee, shall have general charge of all matters of administration and supervision of all arrangements for research and other work undertaken by the Institution or with its funds. He shall prepare and submit to the Board of Trustees and to the Executive



## CARNEGIE INSTITUTION OF WASHINGTON

Committee plans and suggestions for the work of the Institution, shall conduct its general correspondence and the correspondence with applicants for grants and with the special advisers of the Committee, and shall present his recommendations in each case to the Executive Committee for decision. All proposals and requests for grants shall be referred to the President for consideration and report. He shall have power to remove, appoint, and, within the scope of funds made available by the Trustees, provide for compensation of subordinate employees and to fix the compensation of such employees within the limits of a maximum rate of compensation to be established from time to time by the Executive Committee. He shall be *ex officio* a member of the Executive Committee.

2. He shall be the legal custodian of the seal and of all property of the Institution whose custody is not otherwise provided for. He shall sign and execute on behalf of the corporation all contracts and instruments necessary in authorized administrative and research matters and affix the corporate seal thereto when necessary, and may delegate the performance of such acts and other administrative duties in his absence to the Executive Officer. He may execute all other contracts, deeds, and instruments on behalf of the corporation and affix the seal thereto when expressly authorized by the Board of Trustees or Executive Committee. He may, within the limits of his own authorization, delegate to the Executive Officer authority to act as custodian of and affix the corporate seal. He shall be responsible for the expenditure and disbursement of all funds of the Institution in accordance with the directions of the Board and of the Executive Committee, and shall keep accurate accounts of all receipts and disbursements. Following approval by the Executive Committee he shall transmit to the Board of Trustees before its annual meeting in December a written report of the operations and business of the Institution for the preceding fiscal year with his recommendations for work and appropriations for the succeeding calendar year.

3. He shall attend all meetings of the Board of Trustees.

4. There shall be an officer designated Executive Officer who shall be appointed by and hold office at the pleasure of the President, subject to the approval of the Executive Committee. His duties shall be to assist and act for the President as the latter may duly authorize and direct.

5. The President shall retire from office at the end of the calendar year in which he becomes sixty-five years of age.

## ARTICLE IV

### MEETINGS

1. The annual meeting of the Board of Trustees shall be held in the City of Washington, in the District of Columbia, on the first Friday following the second Thursday of December in each year unless the date and place of meeting are otherwise ordered by the Executive Committee.

2. Special meetings of the Board may be called by the Executive Committee by notice served personally upon, or mailed to the usual address of, each Trustee twenty days prior to the meeting.

3. Special meetings shall, moreover, be called in the same manner by the Chairman upon the written request of seven members of the Board.



## BY-LAWS OF THE INSTITUTION

### ARTICLE V

#### COMMITTEES

1. There shall be the following standing Committees, *viz.* an Executive Committee, a Finance Committee, an Auditing Committee, a Nominating Committee, and a Retirement Committee.

2. All vacancies occurring in the Executive Committee, the Finance Committee, the Auditing Committee, the Nominating Committee, and the Retirement Committee shall be filled by the Trustees at the next regular meeting. In case of vacancy in the Finance Committee, the Auditing Committee, the Nominating Committee, or the Retirement Committee, upon request of the remaining members of such committee, the Executive Committee may fill such vacancy by appointment until the next meeting of the Board of Trustees.

3. The terms of all officers and of all members of committees, as provided for herein, shall continue until their successors are elected or appointed.

#### *Executive Committee*

4. The Executive Committee shall consist of the Chairman, Vice-Chairman, and Secretary of the Board of Trustees and the President of the Institution *ex officio* and, in addition, five trustees to be elected by the Board by ballot for a term of three years, who shall be eligible for re-election. Any member elected to fill a vacancy shall serve for the remainder of his predecessor's term.

5. The Executive Committee shall, when the Board is not in session and has not given specific directions, have general control of the administration of the affairs of the corporation and general supervision of all arrangements for administration, research, and other matters undertaken or promoted by the Institution. It shall also submit to the Board of Trustees a printed or typewritten report of each of its meetings, and at the annual meeting shall submit to the Board a report for publication.

6. The Executive Committee shall have power to authorize the purchase, sale, exchange, or transfer of real estate.

#### *Finance Committee*

7. The Finance Committee shall consist of not less than five and not more than six members to be elected by the Board of Trustees by ballot for a term of three years, who shall be eligible for re-election.

8. The Finance Committee shall have custody of the securities of the corporation and general charge of its investments and invested funds, including its investments and invested funds as trustee of any retirement plan for the Institution's staff members and employees, and shall care for and dispose of the same subject to the directions of the Board of Trustees. It shall have power to authorize the purchase, sale, exchange, or transfer of securities and to delegate this power. It shall consider and recommend to the Board from time to time such measures as in its opinion will promote the financial interests of the Institution and of the trust fund under any retirement plan for the Institution's staff members and employees, and shall make a report at each meeting of the Board.

## CARNEGIE INSTITUTION OF WASHINGTON

### *Auditing Committee*

9. The Auditing Committee shall consist of three members to be elected by the Board of Trustees by ballot for a term of three years.

10. Before each annual meeting of the Board of Trustees, the Auditing Committee shall cause the accounts of the Institution for the preceding fiscal year to be audited by public accountants. The accountants shall report to the Committee, and the Committee shall present said report at the ensuing annual meeting of the Board with such recommendations as the Committee may deem appropriate.

### *Nominating Committee*

11. The Nominating Committee shall consist of the Chairman of the Board of Trustees *ex officio* and, in addition, three trustees to be elected by the Board by ballot for a term of three years, who shall not be eligible for re-election until after the lapse of one year. Any member elected to fill a vacancy shall serve for the remainder of his predecessor's term, provided that of the Nominating Committee first elected after adoption of this By-Law one member shall serve for one year, one member shall serve for two years, and one member shall serve for three years, the Committee to determine the respective terms by lot.

12. Sixty days prior to an annual meeting of the Board the Nominating Committee shall notify the Trustees by mail of the vacancies to be filled in membership of the Board. Each Trustee may submit nominations for such vacancies. Nominations so submitted shall be considered by the Nominating Committee, and ten days prior to the annual meeting the Nominating Committee shall submit to members of the Board by mail a list of the persons so nominated, with its recommendations for filling existing vacancies on the Board and its Standing Committees. No other nominations shall be received by the Board at the annual meeting except with the unanimous consent of the Trustees present.

### *Retirement Committee*

13. The Retirement Committee shall consist of three members to be elected by the Board of Trustees by ballot for a term of three years, who shall be eligible for re-election. Any member elected to fill a vacancy shall serve for the remainder of his predecessor's term, provided that of the Retirement Committee first elected after adoption of this By-Law one member shall serve for one year, one member shall serve for two years, and one member shall serve for three years, the Committee to determine the respective terms by lot.

14. The Retirement Committee shall, subject to the directions of the Board of Trustees, be responsible for the maintenance of a retirement plan for staff members and employees of the Institution and act for the Institution in its capacity as trustee under any such plan, except that any matter relating to investments under any such plan shall be the responsibility of the Finance Committee subject to the directions of the Board of Trustees. The Committee shall submit a report to the Board at the annual meeting of the Board.

## BY-LAWS OF THE INSTITUTION

### ARTICLE VI

#### FINANCIAL ADMINISTRATION

1. No expenditure shall be authorized or made except in pursuance of a previous appropriation by the Board of Trustees, or as provided in Article V, paragraph 8, hereof.

2. The fiscal year of the Institution shall commence on the first day of July in each year.

3. The Executive Committee shall submit to the annual meeting of the Board a full statement of the finances and work of the Institution for the preceding fiscal year and a detailed estimate of the expenditures of the succeeding calendar year.

4. The Board of Trustees, at the annual meeting in each year, shall make general appropriations for the ensuing calendar year; but nothing contained herein shall prevent the Board of Trustees from making special appropriations at any meeting.

5. The Executive Committee shall have general charge and control of all appropriations made by the Board. Following the annual meeting each year, the Executive Committee may make allotment of funds for the period from January 1 to termination of the fiscal year on June 30. It may also make allotment of funds for the period from July 1 to December 31 in advance of July 1. The Committee shall, however, have full authority for allotment of available funds to meet necessary expenditures by other methods, if desirable, and transfer of balances to meet special needs. It shall make provision for outstanding obligations and for reversion of unexpended balances at termination of the fiscal year.

6. The securities of the Institution and evidences of property, and funds invested and to be invested, shall be deposited in such safe depository or in the custody of such trust company and under such safeguards as the Finance Committee shall designate, subject to directions of the Board of Trustees. Income of the Institution available for expenditure shall be deposited in such banks or depositories as may from time to time be designated by the Executive Committee.

7. Any trust company entrusted with the custody of securities by the Finance Committee may, by resolution of the Board of Trustees, be made Fiscal Agent of the Institution, upon an agreed compensation, for the transaction of the business coming within the authority of the Finance Committee.

### ARTICLE VII

#### AMENDMENT OF BY-LAWS

1. These by-laws may be amended at any annual or special meeting of the Board of Trustees by a two-thirds vote of the members present, provided written notice of the proposed amendment shall have been served personally upon, or mailed to the usual address of, each member of the Board twenty days prior to the meeting.





## ABSTRACT OF MINUTES OF THE FIFTY-SIXTH MEETING OF THE BOARD OF TRUSTEES

The annual meeting of the Board of Trustees was held in Washington, D. C., on Friday, December 10, 1954. The Chairman, Mr. Root, presided.

The following Trustees were in attendance: James F. Bell, Robert Woods Bliss, Lindsay Bradford, Walter S. Gifford, Crawford H. Greenewalt, Barklie Henry, Ernest O. Lawrence, Alfred L. Loomis, Robert A. Lovett, Keith S. McHugh, Henry S. Morgan, Seeley G. Mudd, William I. Myers, Henning W. Prentis, Jr., David Rockefeller, Elihu Root, Jr., Henry R. Shepley, and Charles P. Taft. The President of the Institution, Vannevar Bush, was present, and Caryl P. Haskins joined the meeting following his election as President to succeed Vannevar Bush.

The minutes of the fifty-fifth meeting were approved.

Reports of the President, the Executive Committee, the Finance Committee, the Committee on Retirement Benefits, the Auditor, the Auditing Committee, and the Nominating Committee, and of the Directors of Departments and Research Associates of the Institution were presented and considered.

The sum of \$1,939,395 was appropriated for the calendar year 1955 for expenditure under the general charge and control of the Executive Committee.

Amendments to the By-Laws of the Institution recommended by the Executive Committee were adopted.

The following officers of the Board were re-elected for a period of three years: Elihu Root, Jr., Chairman, and Robert Woods Bliss, Secretary. Barklie Henry was elected Vice-Chairman of the Board for a period of three years.

Henry S. Morgan and Henry R. Shepley were re-elected members of the Executive Committee for three-year terms, and Lindsay Bradford was elected a member of the Executive Committee for the remainder of the term of Mr. Henry, expiring in 1956, as the Vice-Chairmanship carries with it ex-officio membership in the Executive Committee. Alfred L. Loomis, Henry S. Morgan, and Elihu Root, Jr., were re-elected members of the Finance Committee for three-year terms. David Rockefeller was elected a member of the Auditing Committee to serve for three years, and Keith S. McHugh was elected Chairman of the Auditing Committee. Crawford H. Greenewalt was elected a member of the Nominating Committee for a period of three years, succeeding Henry S. Morgan, and Keith S. McHugh was elected Chairman of the Nominating Committee. A Retirement Committee provided by the amended By-Laws was organized with Lindsay Bradford, Barklie Henry, and Henry S. Morgan as members for one-year, two-year, and three-year terms, the Committee to determine the respective terms by lot. Lindsay Bradford was elected Chairman of the Retirement Committee.

The Board then proceeded to consider the recommendations of the Committee on Succession in the Presidency. After discussion, the report of the Committee was accepted and Dr. Caryl P. Haskins was unanimously elected President of the Carnegie Institution of Washington, effective January 1, 1956.





## REPORT OF THE EXECUTIVE COMMITTEE

FOR THE YEAR ENDED JUNE 30, 1954

*To the Trustees of the Carnegie Institution of Washington:*

GENTLEMEN: In accordance with the provisions of the By-Laws, the Executive Committee submits this report to the annual meeting of the Board of Trustees.

During the fiscal year ended June 30, 1954, the Executive Committee held four meetings, printed reports of which have been mailed to each Trustee and constitute a part of this report.

The detailed record of the activities of the Institution is presented in the reports from the Departments, which are contained in the Year Book, a review of some of the highlights being given in the report of the President. The estimate of expenditures for the calendar year 1955 contained in the report of the President has been considered and approved by the Executive Committee, and the Committee has also provisionally approved and recommends to the Board the proposed budget based thereon.

The Board of Trustees, at its meeting of December 11, 1953, appointed the firm of Haskins & Sells to audit the accounts of the Institution for the fiscal year ending June 30, 1954. The report of the Auditor, including a balance sheet showing assets and liabilities of the Institution on June 30, 1954, together with supporting statements and schedules, is submitted as a part of the report of the Executive Committee.

Terms of the following officers of the Board of Trustees will end at the annual meeting in December: Mr. Root, Chairman of the Board; Mr. Bradford, Vice-Chairman of the Board; and Mr. Bliss, Secretary of the Board. Terms of Messrs. Morgan and Shepley as members of the Executive Committee, of Messrs. Loomis, Morgan, and Root as members of the Finance Committee, and of Mr. Miller, Chairman of the Auditing Committee, will end at the annual meeting. The term of Mr. Morgan, Chairman, as a member of the Nominating Committee will also end at the annual meeting.

Messrs. Bradford, Chairman, Henry, and Morgan were appointed by the Executive Committee as the members of the Committee on Retirement Benefits to serve until action is taken by the Board of Trustees at the annual meeting.

ELIHU ROOT, JR., *Chairman*

*October 21, 1954*



HASKINS & SELLS  
CERTIFIED PUBLIC ACCOUNTANTS

500 EQUITABLE BUILDING  
BALTIMORE 2

### ACCOUNTANTS' CERTIFICATE

*To the Board of Trustees of Carnegie Institution of Washington:*

We have examined the balance sheet of Carnegie Institution of Washington as of June 30, 1954 and the related summaries of current income and expenditures, current funds surplus, current restricted gifts and grants, changes in endowment and other special funds, and changes in investment in real estate and equipment for the year then ended (Exhibits A to F, inclusive). Our examination was made in accordance with generally accepted auditing standards, and included such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances.

In our opinion the accompanying balance sheet and above described summaries (Exhibits A to F, inclusive) present fairly the financial position of the Institution at June 30, 1954 and the results of its operations for the year then ended, in conformity with generally accepted accounting principles applied on a basis consistent with that of the preceding year.

HASKINS & SELLS

*September 1, 1954*

## ASSETS

*Current Funds:*

|   |              |                |  |
|---|--------------|----------------|--|
| Cash .....                                      | \$654,205.02 |                |  |
| Advances:                                       |              |                |  |
| Departmental Research Operations.....           | 10,038.42    |                |  |
| Other .....                                     | 914.41       |                |  |
| Accounts receivable .....                       | 749.69       |                |  |
| Inventory of books.....                         | 85,210.05    |                |  |
| Deferred charges .....                          | 22,825.33    |                |  |
| Due from Endowment and Other Special Funds..... | 307,249.16   | \$1,081,192.08 |  |
|   |              |                |  |

*Endowment and Other Special Funds:*

|  |                 |               |               |
|--|-----------------|---------------|---------------|
| Cash .....   | \$57,149.46     |               |               |
| Securities (valuation based on market quotations<br>at June 30, 1954—\$59,215,093)—Schedule 1: |                 |               |               |
| Bonds .....  | \$24,868,139.99 |               |               |
| Preferred stocks .....   | 3,802,249.99    |               |               |
| Common stocks .....  | 16,827,924.15   | 45,498,314.13 | 45,555,463.59 |
|  |                 |               |               |

*Plant Funds:*

|  |              |                 |  |
|--|--------------|-----------------|--|
| Investment in real estate and equipment—Exhibit F..... | 5,178,564.66 |                 |  |
|  |              |                 |  |
| TOTAL .....  |              | \$51,815,220.33 |  |

## LIABILITIES

*Current Funds:*

|   |              |                |  |
|---|--------------|----------------|--|
| Accounts payable .....  | \$4,041.44   |                |  |
| Reserve for valuation of books and accounts receivable.....     | 85,959.74    |                |  |
| Current Funds Surplus—Exhibit C:                                |              |                |  |
| Appropriated unexpended balances.....                           | \$410,294.76 |                |  |
| General Contingent Fund.....                                    | 518,518.06   | 928,812.82     |  |
|   |              |                |  |
| Unexpended balance of restricted gifts and grants—Exhibit D.... | 62,378.08    | \$1,081,192.08 |  |

*Endowment and Other Special Funds:*

|                               |                 |               |               |
|-------------------------------|-----------------|---------------|---------------|
| Due to Current Funds.....     | \$307,249.16    |               |               |
| Principal of Funds—Exhibit E: |                 |               |               |
| Capital funds .....           | \$42,194,449.16 |               |               |
| Special funds .....           | 3,053,765.27    | 45,248,214.43 | 45,555,463.59 |

*Plant Funds:*

|  |                |                 |  |
|--|----------------|-----------------|--|
| Bequests, gifts, and income invested in plant..... | \$5,126,142.56 |                 |  |
| Harriman Fund—donated land.....                    | 12,352.10      |                 |  |
| Harkavy Fund—donated land.....                     | 2,070.00       |                 |  |
| Hale Fund—Solar Laboratory.....                    | 38,000.00      | 5,178,564.66    |  |
|  |                |                 |  |
| TOTAL .....  |                | \$51,815,220.33 |  |



## EXHIBIT B

SUMMARY OF CURRENT INCOME AND EXPENDITURES  
FOR THE YEAR ENDED JUNE 30, 1954

## CURRENT INCOME:

## Investment income:

|   |                |                |
|---|----------------|----------------|
| Interest and dividends on securities..... | \$1,989,950.25 |                |
| Less: Amortization of bond premiums.....  | 23,632.00      | \$1,966,318.25 |

|                                     |  |           |
|-------------------------------------|--|-----------|
| Market value of stock dividend..... |  | 10,510.50 |
|-------------------------------------|--|-----------|

|   |  |                |
|---|--|----------------|
| TOTAL—SCHEDULE 1.....   |  | \$1,976,828.75 |
| Less: Income added to Special Funds (Exhibit E)—Schedule 1..... |  | 1,545.15       |

|  |  |                |
|--|--|----------------|
| Remainder—Income available for current purposes..... |  | \$1,975,283.60 |
|--|--|----------------|

## Other income:

|                              |            |           |
|------------------------------|------------|-----------|
| Sales of publications.....   | \$5,785.38 |           |
| Dormitory and mess hall..... | 10,496.39  |           |
| Miscellaneous .....          | 3,946.35   | 20,228.12 |

|  |  |           |
|--|--|-----------|
| Restricted gifts and grants utilized for current purposes—Exhibit D..... |  | 68,575.88 |
|--|--|-----------|

|                           |  |                |
|---------------------------|--|----------------|
| TOTAL CURRENT INCOME..... |  | \$2,064,087.60 |
|---------------------------|--|----------------|

## CURRENT EXPENDITURES (including expenditures against appropriations of prior years)—Schedule 4:

|  |              |
|--|--------------|
| Administration .....                             | \$238,301.08 |
| Departmental Research Operations.....            | 1,366,891.23 |
| General Publications .....                       | 34,190.22    |
| Research Projects, Fellowships, Grants, etc..... | 91,579.89    |
| Pension Fund—annuity and insurance.....          | 158,050.52   |

\$1,889,012.94

|                                 |           |
|---------------------------------|-----------|
| Gifts and grants—Exhibit D..... | 68,575.88 |
|---------------------------------|-----------|

|                                 |              |
|---------------------------------|--------------|
| TOTAL CURRENT EXPENDITURES..... | 1,957,588.82 |
|---------------------------------|--------------|

|   |                      |
|---|----------------------|
| EXCESS OF CURRENT INCOME OVER CURRENT EXPENDITURES—EXHIBIT C..... | <u>\$106,498.78*</u> |
|---|----------------------|

## \*SUMMARIZED AS FOLLOWS:

|  |              |
|--|--------------|
| Current investment income in excess of Trustees' authorized appropriations during the fiscal year, credited to General Reserve Fund—Exhibit E..... | \$119,183.10 |
| Market value of stock dividend credited to Special Income Reserve—Exhibit E.....   | 10,510.50    |
| Revertment of unexpended current appropriations to General Contingent Fund—Schedule 4.....   | 57,846.01    |
| Reserved from this year's appropriations for current liabilities and commitments—Schedule 4.....   | 145,856.87   |

|             |              |
|-------------|--------------|
| TOTAL ..... | \$333,396.48 |
|-------------|--------------|

|   |            |                     |
|---|------------|---------------------|
| Less: Amount included in current expenditures, applicable to allotments and unexpended balances from prior years' appropriations—Schedule 4 ..... | 226,897.70 | <u>\$106,498.78</u> |
|---|------------|---------------------|

## EXHIBIT C

SUMMARY OF CURRENT FUNDS SURPLUS  
FOR THE YEAR ENDED JUNE 30, 1954

*Balance, July 1, 1953*..... \$940,852.60

## Additions:

|   |              |                |
|---|--------------|----------------|
| Excess of current income over current expenditures—Exhibit B.....   | \$106,498.78 |                |
| Transfer from Special Funds (General Reserve Fund) to cover<br>miscellaneous expenditures in connection with the building<br>program at Cold Spring Harbor—Exhibit E..... | 11,155.04    | 117,653.82     |
|   | <hr/>        | <hr/>          |
| TOTAL .....   |              | \$1,058,506.42 |

## Deductions:

|   |              |                            |
|---|--------------|----------------------------|
| Transfer to Special Funds—Exhibit E:  |              |                            |
| General Reserve Fund, representing excess of investment income<br>over Trustees' appropriations for current purposes..... | \$119,183.10 |                            |
| Special Income Reserve, representing market value of stock divi-<br>dend received during year.....                        | 10,510.50    | 129,693.60                 |
|   | <hr/>        | <hr/>                      |
| <i>Balance, June 30, 1954, per Schedule 3</i> .....   |              | <u><u>\$928,812.82</u></u> |

## EXHIBIT D

SUMMARY OF CURRENT RESTRICTED GIFTS AND GRANTS  
FOR THE YEAR ENDED JUNE 30, 1954

|   | Unex-<br>pended<br>Balance<br>July 1,<br>1953 | Additions—<br>Gifts and<br>Grants<br>Received | Deductions—<br>Expenditures<br>(Schedule 4) | Unex-<br>pended<br>Balance<br>June 30,<br>1954 |
|---|---|---|---|--|
| Departmental Research Operations:             |   |   |   |  |
| Department of Plant Biology:                  |   |   |   |  |
| Research Corporation .....                    | \$1,125.47                                    | .....   | \$1,125.47                                  | .....  |
| Department of Genetics:                       |   |   |   |  |
| American Cancer Society No. EG 21F.....       | 671.04  | \$7,400.00                                    | 6,244.80                                    | \$1,826.24                                     |
| American Cancer Society No. INSP-72.....      | 4,000.00                                      | .....   | 3,883.28                                    | 116.72   |
| U. S. Public Health Service No. RG-149 C7..   | 3,266.61                                      | 9,405.63                                      | 8,822.11                                    | 3,850.13                                       |
| U. S. Public Health Service No. C-2158 C3..   | 747.98  | 11,998.18                                     | 9,846.99                                    | 2,899.17                                       |
| National Foundation for Infantile Paralysis.. | 600.00  | .....   | 595.00                                      | 5.00   |
| Department of Terrestrial Magnetism:          |   |   |   |  |
| National Science Foundation.....              | .....   | 8,500.00                                      | 8,500.00                                    | .....  |
| Research Projects, Fellowships, etc.:         |   |   |   |  |
| Carnegie Corporation of New York:             |   |   |   |  |
| Biology:                                      |   |   |   |  |
| Point Lobos, California.....                  | 4,245.32                                      | .....   | 2,000.00                                    | 2,245.32                                       |
| Yerkes Laboratories of Primate Biology...     | .....   | 12,000.00                                     | 12,000.00                                   | .....  |
| Geology:                                      |   |   |   |  |
| Tilley, C. E.....                             | 620.63  | .....   | .....                                       | 620.63   |
| History:                                      |   |   |   |  |
| Dantzig, Tobias .....                         | 4,000.00                                      | .....   | 4,000.00                                    | .....  |
| Physiology:                                   |   |   |   |  |
| Russell, G. Oscar.....                        | 1,222.44                                      | .....   | .....                                       | 1,222.44                                       |
| Plant Biology:                                |   |   |   |  |
| Chlorella studies .....                       | 2,479.02                                      | .....   | 2,479.02                                    | .....  |
| Terrestrial Magnetism:                        |   |   |   |  |
| Magnetic polarization of the earth.....       | 2,540.54                                      | .....   | 148.55                                      | 2,391.99                                       |
| Clews, C. J. Birkett.....                     | 6,131.10                                      | .....   | 3,729.41                                    | 2,401.69                                       |
| Telescope image converter.....                | .....   | 50,000.00                                     | 5,201.25                                    | 44,798.75                                      |
| TOTAL .....                                   | <u>\$31,650.15</u>                            | <u>\$99,303.81</u>                            | <u>\$68,575.88</u>                          | <u>\$62,378.08</u>                             |

EXHIBIT E

SUMMARY OF CHANGES IN ENDOWMENT AND OTHER SPECIAL FUNDS FOR THE YEAR ENDED JUNE 30, 1954

|                              | ADDITIONS                               |  |  | DEDUCTIONS  |                                      |            | Balance<br>June 30, 1954 |
|------------------------------|---|--|--|---|--------------------------------------|------------|--------------------------|
|                              | Gifts, Sale<br>of Property<br>and Misc. | Income from<br>Investments<br>Added<br>to Funds<br>(Exhibit B) | Net Realized<br>Gain on<br>Investments<br>(Schedule 2) | Transfer to<br>Current<br>Funds<br>Surplus<br>(Exhibit C) | Expenditures<br>Plant<br>(Exhibit F) | Other      |                          |
| Capital Funds:               |   |  |  |   |                                      |            |                          |
| Endowment Fund               | .....                                   | .....  | .....  | .....   | .....                                | .....      | \$32,000,000.00          |
| Capital Reserve Fund         | .....                                   | .....  | \$814,733.57   | .....   | .....                                | .....      | 10,075,912.56            |
| Colburn Fund                 | .....                                   | .....  | .....  | .....   | .....                                | .....      | 103,310.80               |
| Harkavy Fund                 | \$230.00                                | .....  | .....  | .....   | .....                                | .....      | 3,058.80                 |
| Harriman Fund                | 5,582.30                                | .....  | .....  | .....   | \$5,582.30                           | .....      | .....                    |
| Teeple Fund                  | .....                                   | .....  | .....  | .....   | .....                                | .....      | 10,888.42                |
| Van Gelder Fund              | .....                                   | .....  | .....  | .....   | .....                                | .....      | 1,278.58                 |
| Special Funds:               |   |  |  |   |                                      |            |                          |
| Bickel Fund                  | .....                                   | \$461.49   | .....  | .....   | .....                                | .....      | 11,062.27                |
| General Reserve Fund*        | 1,614.95                                | 119,183.10   | .....  | \$11,155.04   | 83,457.35                            | .....      | 2,746,859.57             |
| George E. Hale Relief Fund   | .....                                   | 171.12   | .....  | .....   | .....                                | .....      | 4,164.44                 |
| Harkavy Fund—Income          | .....                                   | 124.82   | .....  | .....   | .....                                | .....      | 825.64                   |
| Harriet H. Mayor Relief Fund | .....                                   | .....  | .....  | .....   | .....                                | \$1,270.53 | 7,399.47                 |
| Special Income Reserve       | .....                                   | 10,510.50  | .....  | .....   | .....                                | .....      | 212,321.89               |
| Special Purpose Fund         | 10,000.00                               | .....  | .....  | .....   | .....                                | .....      | 52,250.00                |
| Woloff Fund                  | .....                                   | 787.72   | .....  | .....   | .....                                | .....      | 18,881.99                |
| TOTAL                        | \$17,427.25                             | \$131,238.75   | \$814,733.57   | \$11,155.04   | \$89,039.65                          | \$1,270.53 | \$45,248,214.43          |

\* To cover the balance of the cost of completing the building program at Cold Spring Harbor, in excess of the Harriman and Lecture Hall Funds available for this purpose, the Trustees at their December 14, 1951 meeting appropriated an amount not to exceed \$350,000.00 from the General Reserve Fund. \$287,059.56 has been charged against this authorization, including \$94,612.39 during the current fiscal year, of which \$83,457.35 was transferred to Plant Funds and \$11,155.04 was transferred to the Department of Genetics for miscellaneous repairs and alterations. Outstanding contractual commitments for the building program at Cold Spring Harbor at June 30, 1954 aggregated approximately \$4,000.00.



|                                     | CLASSIFICATION OF JUNE 30, 1954 BALANCE |                           |                            |                          |                          |                         |              |                        |
|-------------------------------------|---|---------------------------|----------------------------|--------------------------|--------------------------|-------------------------|--------------|------------------------|
|                                     | Balance<br>July 1, 1953                 | Additions<br>(see Note A) | Deductions<br>(see Note B) | Balance<br>June 30, 1954 | Buildings<br>and Grounds | Laboratory<br>Apparatus | Library      | Operating<br>Equipment |
| DEPARTMENTS OF RESEARCH:            |   |                           |                            |                          |                          |                         |              |                        |
| Department of Plant Biology         |   |                           |                            |                          |                          |                         |              |                        |
| Stanford, California .....          | \$162,623.06                            | \$2,358.49                | \$8,689.94                 | \$156,291.61             | \$71,897.68              | \$42,080.46             | \$26,191.86  | \$16,121.61            |
| Department of Genetics              |   |                           |                            |                          |                          |                         |              |                        |
| Long Island, New York.....          | 1,113,139.07                            | 95,474.15                 | 12,538.55                  | 1,196,074.67             | 1,020,656.10             | 75,339.73               | 73,724.33    | 26,354.51              |
| Geophysical Laboratory              |   |                           |                            |                          |                          |                         |              |                        |
| Washington, D. C.....               | 436,776.87                              | 38,767.30                 | 20,283.56                  | 455,260.61               | 170,383.79               | 195,423.80              | 43,314.42    | 46,138.60              |
| Department of Archaeology           |   |                           |                            |                          |                          |                         |              |                        |
| Cambridge, Massachusetts .....      | 21,556.55                               | 325.17                    | 1,832.71                   | 20,049.01                | .....                    | .....                   | 1,280.26     | 18,768.75              |
| Mount Wilson Observatory            |   |                           |                            |                          |                          |                         |              |                        |
| Pasadena, California .....          | 1,654,847.42                            | 14,284.55                 | 9,218.97                   | 1,659,913.00             | 271,597.62               | 1,241,677.39            | 78,793.82    | 67,844.17              |
| Department of Terrestrial Magnetism |   |                           |                            |                          |                          |                         |              |                        |
| Washington, D. C.....               | 773,536.40                              | 37,656.66                 | 8,944.24                   | 802,248.82               | 401,418.69               | 284,015.35              | 44,063.71    | 72,751.07              |
| Department of Embryology            |   |                           |                            |                          |                          |                         |              |                        |
| Baltimore, Maryland .....           | 54,106.61                               | 5,362.98                  | 3,660.93                   | 55,808.66                | .....                    | 42,181.05               | 9,054.36     | 4,573.25               |
| Total Departments of Research. .... | \$4,216,585.98                          | \$194,229.30              | \$65,168.90                | \$4,345,646.38           | \$1,935,953.88           | \$1,880,717.78          | \$276,422.76 | \$252,551.96           |
| OFFICE OF ADMINISTRATION            |   |                           |                            |                          |                          |                         |              |                        |
| Washington, D. C.....               | 832,577.46                              | 979.52                    | 638.70                     | 832,918.28               | 797,632.96               | .....                   | .....        | 35,285.32              |
| TOTAL .....                         | \$5,049,163.44                          | \$195,208.82              | \$65,807.60                | \$5,178,564.66           | \$2,733,586.84           | \$1,880,717.78          | \$276,422.76 | \$287,837.28           |

NOTES:

- A. Additions during year provided from following:  
Current expenditures for equipment—Schedule 4 ..... \$54,545.82  
Expenditures charged to Endowment and Other Special Funds for building program at Cold Spring Harbor—Exhibit E ..... 89,039.65  
Cost of equipment not capitalized in prior year:  
Geophysical Laboratory ..... 13,641.30  
Cost of constructed equipment capitalized:  
Mount Wilson Observatory ..... 10,232.05  
Department of Terrestrial Magnetism ..... 27,750.00  
Total, as above ..... \$195,208.82
- B. Includes \$53,608.91 representing cost of equipment acquired during period January 1, 1925 to January 1, 1930, written off in accordance with authorization passed by the Executive Committee at its meeting on May 20, 1948.



SCHEDULE 1

ENDOWMENT AND OTHER SPECIAL FUNDS INVESTMENTS AS OF  
JUNE 30, 1954 AND INCOME THEREFROM DURING THE YEAR

|                                   | Book Value      | Market Value | Per Cent of<br>Total Investments |                 | Income<br>Received |
|-----------------------------------|-----------------|--------------|----------------------------------|-----------------|--------------------|
|                                   |                 |              | Book<br>Value                    | Market<br>Value |                    |
| Bonds:                            |                 |              |                                  |                 |                    |
| United States Government.....     | \$10,094,128.64 | \$10,250,131 | 22.16%                           | 17.29%          | \$227,431.74       |
| Foreign and International Bank..  | 1,024,432.27    | 1,030,313    | 2.25%                            | 1.74%           | 40,751.49          |
| Public Utility .....              | 3,257,135.75    | 3,219,510    | 7.15%                            | 5.43%           | 110,437.07         |
| Communication .....               | 964,514.35      | 960,750      | 2.12%                            | 1.62%           | 33,644.84          |
| Railroad .....                    | 629,864.03      | 670,725      | 1.38%                            | 1.13%           | 27,421.64          |
| Railroad Equipment Trust.....     | 1,075,851.97    | 1,085,002    | 2.36%                            | 1.83%           | 23,708.16          |
| Industrial and Miscellaneous .... | 7,822,212.98    | 7,797,100    | 17.17%                           | 13.16%          | 189,541.05         |
| Total bonds .....                 | \$24,868,139.99 | \$25,013,531 | 54.59%                           | 42.20%          | \$652,935.99 *     |
| Stocks:                           |                 |              |                                  |                 |                    |
| Preferred .....                   | \$3,802,249.99  | \$3,714,460  | 8.35%                            | 6.27%           | \$157,671.20       |
| Common .....                      | 16,827,924.15   | 30,487,102   | 36.94%                           | 51.43%          | 1,166,221.56 †     |
| Total stocks .....                | \$20,630,174.14 | \$34,201,562 | 45.29%                           | 57.70%          | \$1,323,892.76     |
| Cash .....                        | \$57,149.46     | \$57,149     | .12%                             | .10%            |                    |
| TOTAL .....                       | \$45,555,463.59 | \$59,272,242 | 100.00%                          | 100.00%         | \$1,976,828.75 ‡   |

\* After deducting bond premium amortization of \$23,632.00.

† Includes \$10,510.50 representing market value of a stock dividend received.

‡ Income received allocated to Endowment and Other Special Funds as follows:

|  |                |
|--|----------------|
| Funds, the income from which may be used for current general purposes—Exhibit B... | \$1,975,283.60 |
| Funds, the income from which is restricted to specific purposes—Exhibit E:         |                |
| Bickel Fund .....  | \$461.49       |
| George E. Hale Relief Fund.....  | 171.12         |
| Harkavy Fund .....   | 124.82         |
| Woloff Fund .....  | 787.72         |
|  | 1,545.15       |
| TOTAL .....  | \$1,976,828.75 |

## SCHEDULE 2

## SCHEDULE OF SECURITIES

| Principal amount                     | Description   | Ma-<br>turity | Book<br>value          | Approximate<br>market<br>value |
|--------------------------------------|---|---------------|------------------------|--------------------------------|
| UNITED STATES GOVERNMENT BONDS       |   |               |                        |                                |
| \$225,000                            | U. S. of America Treasury Notes 1 $\frac{7}{8}$ s.....                              | 1959          | \$224,929.69           | \$226,055                      |
| 1,700,000                            | U. S. of America Treasury 2s.....   | 1954-52       | 1,700,000.00           | 1,712,219                      |
| 2,350,000                            | U. S. of America Treasury 2 $\frac{1}{4}$ s.....                                    | 1962-59       | 2,380,015.43*          | 2,382,313                      |
| 1,500,000                            | U. S. of America Treasury 2 $\frac{1}{2}$ s.....                                    | 1958          | 1,498,089.77           | 1,550,625                      |
| 1,250,000                            | U. S. of America Treasury 2 $\frac{1}{2}$ s.....                                    | 1961          | 1,250,000.00           | 1,280,469                      |
| 800,000                              | U. S. of America Treasury 2 $\frac{3}{4}$ s.....                                    | 1961          | 800,000.00             | 832,000                        |
| 1,000,000                            | U. S. of America Treasury 2 $\frac{3}{4}$ s.....                                    | 1980-75       | 991,093.75             | 993,750                        |
| 400,000                              | U. S. of America Treasury 3 $\frac{1}{4}$ s.....                                    | 1983-78       | 400,000.00             | 441,000                        |
| 50,000                               | U. S. of America Savings Series "G" 2 $\frac{1}{2}$ s.....                          | 1954          | 50,000.00              | 50,000                         |
| 100,000                              | U. S. of America Savings Series "G" 2 $\frac{1}{2}$ s.....                          | 1955          | 100,000.00             | 99,200                         |
| 100,000                              | U. S. of America Savings Series "G" 2 $\frac{1}{2}$ s.....                          | 1956          | 100,000.00             | 98,200                         |
| 100,000                              | U. S. of America Savings Series "G" 2 $\frac{1}{2}$ s.....                          | 1957          | 100,000.00             | 97,600                         |
| 100,000                              | U. S. of America Savings Series "G" 2 $\frac{1}{2}$ s.....                          | 1958          | 100,000.00             | 97,000                         |
| 100,000                              | U. S. of America Savings Series "G" 2 $\frac{1}{2}$ s.....                          | 1959          | 100,000.00             | 96,100                         |
| 100,000                              | U. S. of America Savings Series "G" 2 $\frac{1}{2}$ s.....                          | 1960          | 100,000.00             | 95,200                         |
| 200,000                              | U. S. of America Savings Series "K" 2.76s.....                                      | 1966          | 200,000.00             | 198,400                        |
| <u>\$10,075,000</u>                  | <i>Total U. S. Government.....</i>  |               | <u>\$10,094,128.64</u> | <u>\$10,250,131</u>            |
| FOREIGN AND INTERNATIONAL BANK BONDS |   |               |                        |                                |
| \$300,000                            | Aluminum Company of Canada, Ltd., S. F. Deb.<br>3 $\frac{7}{8}$ s Guar. ....        | 1970          | \$303,990.16*          | \$315,750                      |
| 100,000                              | Canadian National Ry. Co., 4 $\frac{1}{2}$ s Guar.....                              | 1957          | 112,000.00             | 108,375                        |
| 125,000                              | International Bank for Reconstruction and Develop-<br>ment, 3s .....                | 1976          | 125,000.00             | 124,375                        |
| 125,000                              | International Bank for Reconstruction and Develop-<br>ment, 3 $\frac{3}{8}$ s ..... | 1975          | 123,125.00             | 127,813                        |
| 150,000                              | Noranda Mines Ltd., S. F. Deb. 4 $\frac{3}{4}$ s.....                               | 1968          | 154,197.11*            | 159,000                        |
| 200,000                              | Shawinigan Water & Power Co., 1st Mtg. & Coll.<br>Tr. S. F. 3s Series "M".....      | 1971          | 206,120.00*            | 195,000                        |
| <u>\$1,000,000</u>                   | <i>Total Foreign and International Bank.....</i>                                    |               | <u>\$1,024,432.27</u>  | <u>\$1,030,313</u>             |
| PUBLIC UTILITY BONDS                 |   |               |                        |                                |
| \$100,000                            | American Gas & Electric Co., Serial Note 2 $\frac{1}{2}$ s....                      | 1955          | \$100,615.81*          | \$100,250                      |
| 125,000                              | Columbia Gas System, Inc., Deb. 3s.....   | 1975          | 127,862.39*            | 120,000                        |
| 237,000                              | Columbus & Southern Ohio Electric Co., 1st Mtg.<br>3 $\frac{1}{4}$ s .....          | 1970          | 247,343.30*            | 246,480                        |
| 300,000                              | Consolidated Natural Gas Co., Deb. 2 $\frac{3}{4}$ s.....                           | 1968          | 300,601.30*            | 302,625                        |
| 300,000                              | Consumers Power Co., 1st Mtg. 3 $\frac{3}{4}$ s.....                                | 1983          | 303,691.95*            | 315,750                        |
| 200,000                              | Minnesota Power & Light Co., 1st Mtg. 3 $\frac{1}{8}$ s.....                        | 1975          | 203,487.99*            | 199,000                        |
| 100,000                              | Northern Natural Gas Co., Serial Deb. 2 $\frac{7}{8}$ s.....                        | 1955          | 100,595.56*            | 100,375                        |
| 100,000                              | Ohio Power Co., 1st Mtg. 3 $\frac{1}{4}$ s.....                                     | 1968          | 101,500.00             | 103,250                        |
| 200,000                              | Pacific Gas and Electric Co., 1st & Ref. Mtg. 3 $\frac{1}{8}$ s..                   | 1984          | 201,750.00             | 200,750                        |
| 200,000                              | Panhandle Eastern Pipe Line Co., Serial Deb. 2 $\frac{3}{4}$ s.                     | 1961-62       | 201,432.85*            | 199,750                        |
| 100,000                              | Panhandle Eastern Pipe Line Co., S. F. Deb. 3 $\frac{1}{4}$ s..                     | 1973          | 101,372.82*            | 100,250                        |
| 50,000                               | Philadelphia Electric Co., 1st & Ref. Mtg. 2 $\frac{7}{8}$ s.....                   | 1978          | 49,687.50              | 49,500                         |
| 207,000                              | Philadelphia Electric Power Co., 1st Mtg. 2 $\frac{7}{8}$ s....                     | 1975          | 210,667.82*            | 199,755                        |
| 200,000                              | Public Service Co. of Indiana, Inc., 1st Mtg. 3 $\frac{1}{8}$ s..                   | 1975          | 203,535.87*            | 201,500                        |
| 45,000                               | Puget Sound Power & Light Co., 1st Mtg. 4 $\frac{1}{4}$ s....                       | 1972          | 46,140.61*             | 47,700                         |
| 210,000                              | Tennessee Gas & Transmission Co., 1st Mtg. Pipe<br>Line 2 $\frac{3}{4}$ s .....     | 1966          | 211,890.00*            | 199,500                        |
| 191,000                              | Tennessee Gas Transmission Co., 1st Mtg. Pipe Line<br>3s .....                      | 1969          | 195,331.06*            | 186,225                        |
| 83,000                               | Tennessee Gas Transmission Co., S. F. Deb. 4 $\frac{7}{8}$ s..                      | 1971          | 84,628.92*             | 87,150                         |
| 265,000                              | United Gas Corp., 1st Mtg. & Coll. Tr. 2 $\frac{3}{4}$ s.....                       | 1967          | 265,000.00             | 259,700                        |
| <u>\$3,213,000</u>                   | <i>Total Public Utility.....</i>  |               | <u>\$3,257,135.75</u>  | <u>\$3,219,510</u>             |

\* After deduction for amortization of premiums on bonds purchased subsequent to January 1, 1940.

SCHEDULE OF SECURITIES—Continued

| Principal<br>amount                | Description  | Ma-<br>turity | Book<br>value          | Approximate<br>market<br>value |
|------------------------------------|--|---------------|------------------------|--------------------------------|
| COMMUNICATION BONDS                |  |               |                        |                                |
| \$150,000                          | American Telephone & Telegraph Co., Deb. 2¾s.            | 1975          | \$151,856.25*          | \$144,000                      |
| 200,000                            | Mountain States Telephone & Telegraph Co., Deb. 3½s      | 1978          | 201,330.00*            | 201,000                        |
| 100,000                            | New York Telephone Co., Ref. Mtg. 3½s                    | 1978          | 101,134.14*            | 103,250                        |
| 200,000                            | Pacific Telephone & Telegraph Co., Deb. 3¼s              | 1978          | 204,193.96*            | 208,750                        |
| 300,000                            | Southwestern Bell Telephone Co., Deb. 3½s                | 1983          | 306,000.00*            | 303,750                        |
| <u>\$950,000</u>                   | <i>Total Communication</i>                               |               | <u>\$964,514.35</u>    | <u>\$960,750</u>               |
| RAILROAD BONDS                     |  |               |                        |                                |
| \$100,000                          | Chesapeake & Ohio Ry. Co., Gen. Mtg. 4½s                 | 1992          | \$99,464.29            | \$126,625                      |
| 276,000                            | Fort Worth & Denver Rwy. Co., 1st Mtg. 4¾s               | 1982          | 278,577.07*            | 289,800                        |
| 100,000                            | Pennsylvania R. R. Co., Cons. Mtg. 4½s                   | 1960          | 104,662.50             | 107,125                        |
| 140,000                            | Western Maryland Rwy. Co., 1st Mtg. 4½s                  | 1976          | 147,160.17*            | 147,175                        |
| <u>\$616,000</u>                   | <i>Total Railroad</i>                                    |               | <u>\$629,864.03</u>    | <u>\$670,725</u>               |
| RAILROAD EQUIPMENT TRUST BONDS     |  |               |                        |                                |
| \$150,000                          | Chesapeake & Ohio Ry. Co., Eq. Tr. 2s Guar.              | 1956-58       | \$146,340.34           | \$149,438                      |
| 300,000                            | Chicago Burlington & Quincy R. R. Co., Eq. Tr. 2¼s Guar. | 1958-63       | 292,507.12             | 294,125                        |
| 100,000                            | Great Northern Railway Co., Eq. Tr. 2s Guar.             | 1960-61       | 98,538.91              | 96,438                         |
| 150,000                            | Pennsylvania R. R. Co., Eq. Tr. 2¾s Guar.                | 1958-62       | 146,358.96             | 145,688                        |
| 100,000                            | Southern Pacific Co., Eq. Tr. 2½s Guar.                  | 1956&59       | 99,926.85*             | 99,625                         |
| 150,000                            | Southern Pacific Co., Eq. Tr. 2½s Guar.                  | 1956-58       | 146,251.10             | 149,688                        |
| 150,000                            | Southern Railway Co., Eq. Tr. 2½s Guar.                  | 1956-58       | 145,928.69             | 150,000                        |
| <u>\$1,100,000</u>                 | <i>Total Railroad Equipment Trust</i>                    |               | <u>\$1,075,851.97</u>  | <u>\$1,085,002</u>             |
| INDUSTRIAL AND MISCELLANEOUS BONDS |  |               |                        |                                |
| \$200,000                          | Allied Chemical and Dye Corp., Deb. 3½s                  | 1978          | \$198,000.00           | \$209,500                      |
| 100,000                            | Aluminum Company of America, S. F. Deb. 3s               | 1979          | 100,000.00             | 100,250                        |
| 200,000                            | Aluminum Company of America, S. F. 3½s                   | 1964          | 200,000.00             | 205,500                        |
| 187,000                            | American Tobacco Co., Deb. 3s                            | 1969          | 189,081.89*            | 184,195                        |
| 234,000                            | Bristol Myers Co., Deb. 3s                               | 1968          | 234,807.62*            | 234,585                        |
| 300,000                            | C. I. T. Financial Corp., Deb. 2¾s                       | 1959          | 300,000.00*            | 299,625                        |
| 150,000                            | Dow Chemical Co., Deb. 2.35s                             | 1961          | 150,443.19*            | 148,500                        |
| 130,000                            | Dow Chemical Co., Conv. Sub. Deb. 3s                     | 1982          | 131,857.66*            | 138,288                        |
| 153,000                            | Food Machinery Corp., S. F. Deb. 2½s                     | 1962          | 152,308.98             | 145,350                        |
| 180,000                            | General Motors Acceptance Corp., Deb. 4s                 | 1958          | 180,000.00             | 185,625                        |
| 500,000                            | General Motors Corporation, Deb. 3¼s                     | 1979          | 502,500.00             | 519,375                        |
| 275,000                            | Goodrich (B. F.) Company, 1st Mtg. 2¾s                   | 1965          | 275,723.73*            | 275,688                        |
| 247,000                            | P. Lorillard Co., Deb. 3s                                | 1963          | 251,604.42*            | 247,000                        |
| 295,000                            | National Dairy Products Corp., Deb. 2¾s                  | 1970          | 298,274.02*            | 292,419                        |
| 488,000                            | Phillips Petroleum Co., S. F. Deb. 2¾s                   | 1964          | 492,400.75*            | 488,000                        |
| 200,000                            | Phillips Petroleum Co., Conv. S. F. Deb. 3.70s           | 1983          | 206,517.24*            | 220,500                        |
| 125,000                            | Pittsburgh Plate Glass Co., S. F. Deb. 3s                | 1967          | 125,000.00             | 127,031                        |
| 150,000                            | Quaker Oats Co., Deb. 2¾s                                | 1964          | 148,922.50             | 150,000                        |
| 300,000                            | Seagram (Joseph E.) & Sons, Inc., Deb. 2½s               | 1966          | 298,500.00             | 285,750                        |
| 300,000                            | Service Pipe Line Co., S. F. Deb. 3.20s                  | 1982          | 300,000.00             | 309,000                        |
| 500,000                            | Shell Union Oil Corp., Deb. 2½s                          | 1971          | 503,437.07*            | 478,125                        |
| 350,000                            | Sinclair Oil Corp., Conv. Sub. Deb. 3¼s                  | 1983          | 357,811.25             | 370,563                        |
| 500,000                            | Socony-Vacuum Oil Co., Inc., Deb. 2½s                    | 1976          | 489,528.75             | 476,250                        |
| 100,000                            | Standard Oil Co. (Ind.), Conv. Deb. 3½s                  | 1982          | 101,238.16*            | 106,750                        |
| 300,000                            | Swift & Co., Deb. 2¾s                                    | 1972          | 301,276.49*            | 294,000                        |
| 500,000                            | Texas Corporation, Deb. 3s                               | 1965          | 517,859.55*            | 514,375                        |
| 346,000                            | Union Oil Company of California, Deb. 2¾s                | 1970          | 354,731.83*            | 339,080                        |
| 57,000                             | Union Oil Company of California, Conv. Sub. Deb. 3½s     | 1972          | 57,000.00              | 60,776                         |
| 400,000                            | Westinghouse Electric Corporation, Deb. 2¾s              | 1971          | 403,387.88*            | 391,000                        |
| <u>\$7,767,000</u>                 | <i>Total Industrial and Miscellaneous</i>                |               | <u>\$7,822,212.98</u>  | <u>\$7,797,100</u>             |
| <u>\$24,721,000</u>                | <i>BONDS—Funds Invested</i>                              |               | <u>\$24,868,139.99</u> | <u>\$25,013,531</u>            |

\* After deduction for amortization of premiums on bonds purchased subsequent to January 1, 1940.



# SCHEDULE OF SECURITIES—Continued

| Number<br>of<br>shares | Description   | Book<br>value  | Approximate<br>market<br>value |
|------------------------|---|----------------|--------------------------------|
| PREFERRED STOCKS       |   |                |                                |
| 400                    | Air Reduction Company, Inc., 4.50% Cum. Conv. Pref.....       | \$41,195.04    | \$44,000                       |
| 1,500                  | Appalachian Electric Power Co., 4½% Cum. Pref.....            | 159,000.00     | 160,500                        |
| 2,000                  | Armstrong Cork Co., \$3.75 Cum. Pref.....                     | 205,500.00     | 194,000                        |
| 1,500                  | Bethlehem Steel Corp., 7% Cum. Pref.....                      | 183,637.50     | 238,500                        |
| 500                    | Case (J. I.) Co., 7% Cum. Pref.....                           | 62,225.00      | 58,500                         |
| 600                    | Cleveland Electric Illuminating Co., \$4.50 Cum. Pref.....    | 68,112.25      | 65,250                         |
| 1,900                  | Consolidated Edison Co. of N. Y., Inc., \$5.00 Cum. Pref..... | 202,815.50     | 207,338                        |
| 1,125                  | Continental Can Co., Inc., \$3.75 Cum. Pref.....              | 115,312.50     | 109,969                        |
| 600                    | Corn Products Refining Co., 7% Cum. Pref.....                 | 110,335.18     | 105,000                        |
| 2,075                  | duPont (E. I.) de Nemours & Co., \$4.50 Cum. Pref.....        | 235,401.89     | 247,703                        |
| 1,000                  | El Paso Natural Gas Co., 4.10% Cum. Pref.....                 | 111,442.21     | 95,000                         |
| 1,500                  | General Foods Corp., \$3.50 Cum. Pref.....                    | 150,750.00     | 141,000                        |
| 1,500                  | General Motors Corp., \$5.00 Cum. Pref.....                   | 187,937.50     | 187,125                        |
| 1,000                  | General Shoe Corporation, \$3.50 Cum. Pref. "A".....          | 102,250.00     | 88,000                         |
| 1,000                  | Grant (W. T.) Co., 3¾% Cum. Pref.....                         | 100,447.91     | 96,500                         |
| 300                    | Merck & Co., Inc., \$4.00 Conv. Cum. 2nd Pref.....            | 31,200.00      | 30,900                         |
| 800                    | National Distillers Products Corp., 4¼% Cum. Conv. Pref....   | 80,000.00      | 73,200                         |
| 2,000                  | Niagara Mohawk Power Corp., 3.60% Cum. Pref.....              | 207,990.00     | 178,500                        |
| 1,300                  | Ohio Power Co., 4½% Cum. Pref.....                            | 144,630.02     | 141,050                        |
| 1,500                  | Pacific Telephone and Telegraph Co., 6% Cum. Pref.....        | 235,220.75     | 217,125                        |
| 1,000                  | Panhandle Eastern Pipe Line Co., 4% Cum. Pref.....            | 104,166.68     | 102,000                        |
| 900                    | Pillsbury Mills, Inc., \$4.00 Cum. Pref.....                  | 96,949.80      | 90,900                         |
| 2,000                  | Reynolds (R. J.) Tobacco Co., 3.60% Cum. Pref.....            | 199,683.75     | 156,500                        |
| 750                    | Sherwin-Williams Co., 4% Cum. Pref.....                       | 82,662.66      | 78,375                         |
| 1,300                  | Standard Oil Co. of Ohio, 3¾% Cum. Pref. "A".....             | 139,976.28     | 129,350                        |
| 3,100                  | U. S. Steel Corp., 7% Cum. Pref.....                          | 443,407.57     | 478,175                        |
| 33,150                 | Total Preferred Stocks.....                                   | \$3,802,249.99 | \$3,714,460                    |

|               |  |             |           |
|---------------|--|-------------|-----------|
| COMMON STOCKS |  |             |           |
| 2,000         | Allied Chemical & Dye Corp.....                  | \$96,175.97 | \$183,500 |
| 5,300         | Aluminium Limited .....                          | 221,852.22  | 337,213   |
| 19,360        | American Can Company.....                        | 445,351.97  | 914,760   |
| 2,600         | American Cyanamid Co.....                        | 161,924.48  | 117,000   |
| 10,250        | American Gas and Electric Company.....           | 214,294.48  | 379,250   |
| 5,000         | American Telephone & Telegraph Co.....           | 737,124.15  | 834,375   |
| 3,000         | Anderson, Clayton & Co.....                      | 164,768.84  | 117,000   |
| 4,700         | Armstrong Cork Company.....                      | 231,516.80  | 344,275   |
| 2,800         | Atchison, Topeka and Santa Fe Rwy. Co.....       | 232,758.69  | 310,100   |
| 7,500         | C. I. T. Financial Corporation.....              | 151,369.19  | 279,375   |
| 5,000         | Carrier Corporation .....                        | 202,162.77  | 273,750   |
| 3,000         | Caterpillar Tractor Co.....                      | 151,185.22  | 165,750   |
| 2,000         | Chase National Bank of the City of New York..... | 71,361.04   | 92,000    |
| 60            | Christiana Securities Co.....                    | 356,143.00  | 540,000   |
| 5,400         | Cleveland Electric Illuminating Company.....     | 227,773.54  | 325,350   |
| 6,500         | Consolidated Edison Company of N. Y., Inc.....   | 253,066.41  | 287,625   |
| 4,000         | Consumers Power Co.....                          | 145,974.04  | 177,000   |
| 2,300         | Continental Can Company, Inc.....                | 106,779.99  | 162,438   |
| 5,373         | Continental Insurance Co.....                    | 203,794.08  | 478,197   |
| 12,200        | Continental Oil Co. of Delaware.....             | 239,598.64  | 796,050   |
| 1,000         | Corning Glass Works.....                         | 59,631.83   | 111,250   |
| 3,500         | Deere & Company.....                             | 117,181.35  | 104,125   |
| 6,100         | Delaware Power & Light Company.....              | 128,803.87  | 177,663   |
| 4,200         | Dow Chemical Company.....                        | 154,131.30  | 171,675   |
| 4,800         | duPont (E. I.) de Nemours & Co.....              | 195,905.06  | 643,200   |
| 7,980         | Eastman Kodak Co.....                            | 209,388.59  | 484,785   |
| 170           | Fiber Products, Inc.....                         | 1,036.31    | 1,381     |
| 4,344         | Fireman's Fund Insurance Co.....                 | 128,858.78  | 270,414   |

(Concluded on following page)



SCHEDULE OF SECURITIES—*Continued*

| Number<br>of<br>shares                | Description  | Book<br>value          | Approximate<br>market<br>value |
|---------------------------------------|--|------------------------|--------------------------------|
| <b>COMMON STOCKS—<i>Concluded</i></b> |  |                        |                                |
| 42,000                                | General Electric Co.....                               | \$866,162.00           | \$1,932,000                    |
| 4,000                                 | General Foods Corporation .....                        | 167,302.84             | 286,000                        |
| 7,800                                 | General Motors Corporation.....                        | 268,473.03             | 567,450                        |
| 4,700                                 | Goodrich (B. F.) Company.....                          | 275,643.32             | 441,800                        |
| 2,000                                 | Guaranty Trust Co. of N. Y.....                        | 98,003.91              | 132,500                        |
| 14,800                                | Gulf Oil Corp.....                                     | 307,802.06             | 808,450                        |
| 10,625                                | Gulf States Utilities Co.....                          | 223,782.34             | 343,984                        |
| 5,000                                 | Halliburton Oil Well Cementing Co.....                 | 119,534.11             | 231,250                        |
| 1,000                                 | Hartford Fire Insurance Co.....                        | 53,952.55              | 167,000                        |
| 10,000                                | Humble Oil & Refining Co.....                          | 166,903.20             | 642,500                        |
| 2,500                                 | Illinois Power Co.....                                 | 97,697.35              | 116,250                        |
| 9,528                                 | Insurance Company of North America .....               | 243,482.20             | 938,508                        |
| 4,446                                 | International Business Machines Corp.....              | 213,004.45             | 1,333,800                      |
| 5,000                                 | International Nickel Co. of Canada, Ltd.....           | 185,533.15             | 207,500                        |
| 4,400                                 | International Paper Company .....                      | 218,436.18             | 325,600                        |
| 3,600                                 | Johns-Manville Corp. ....                              | 188,607.00             | 271,800                        |
| 9,000                                 | Kennecott Copper Corporation.....                      | 459,524.19             | 734,625                        |
| 5,000                                 | Kimberly-Clark Corporation .....                       | 227,813.88             | 342,500                        |
| 7,000                                 | Kresge (S. S.) Company.....                            | 220,699.69             | 234,500                        |
| 1,280                                 | Mellon National Bank and Trust Company.....            | 67,193.07              | 110,080                        |
| 8,000                                 | Mercantile Stores Company, Inc.....                    | 174,200.45             | 143,000                        |
| 11,925                                | Merck & Co., Inc.....                                  | 223,709.20             | 214,650                        |
| 7,000                                 | Middle South Utilities, Inc. ....                      | 168,660.65             | 208,250                        |
| 5,500                                 | Minneapolis-Honeywell Regulator Co.....                | 127,075.73             | 533,500                        |
| 3,000                                 | Monsanto Chemical Co.....                              | 126,552.58             | 271,500                        |
| 2,300                                 | National City Bank of New York.....                    | 88,539.38              | 121,900                        |
| 10,000                                | Newberry (J. J.) Co.....                               | 132,451.57             | 341,250                        |
| 8,000                                 | North American Co.....                                 | 140,635.05             | 190,000                        |
| 3,300                                 | Ohio Edison Co.....                                    | 105,150.00             | 141,900                        |
| 7,000                                 | Penney (J. C.) Co.....                                 | 248,413.74             | 614,250                        |
| 1,000                                 | Peoples Gas Light and Coke Company.....                | 106,350.00             | 154,500                        |
| 2,400                                 | Phelps Dodge Corporation.....                          | 71,057.69              | 97,200                         |
| 7,800                                 | Pittsburgh Plate Glass Co.....                         | 251,672.40             | 443,625                        |
| 4,200                                 | Procter & Gamble Co.....                               | 177,227.28             | 365,400                        |
| 5,200                                 | Scott Paper Co.....                                    | 137,909.13             | 509,600                        |
| 2,600                                 | Seaboard Oil Co.....                                   | 229,104.22             | 282,100                        |
| 11,000                                | Sears, Roebuck & Co.....                               | 251,140.45             | 710,875                        |
| 1,250                                 | Security-First National Bank of Los Angeles.....       | 35,039.88              | 63,750                         |
| 11,220                                | Shell Oil Company.....                                 | 413,016.26             | 524,535                        |
| 4,800                                 | Sherwin-Williams Co. ....                              | 294,227.78             | 430,800                        |
| 9,900                                 | Socony-Vacuum Oil Co., Inc.....                        | 300,464.13             | 426,938                        |
| 5,800                                 | Southern California Edison Company .....               | 208,276.33             | 253,750                        |
| 4,500                                 | Southern Railway Co.....                               | 218,508.81             | 253,688                        |
| 5,000                                 | Standard Oil Co. of Indiana.....                       | 181,297.93             | 389,375                        |
| 5,156                                 | Standard Oil Co. of New Jersey.....                    | 169,569.68             | 444,061                        |
| 6,200                                 | Texas Company .....                                    | 163,636.60             | 412,300                        |
| 5,500                                 | Union Carbide & Carbon Corp.....                       | 172,416.63             | 473,000                        |
| 1,800                                 | Union Pacific R. R. Co.....                            | 206,185.36             | 242,100                        |
| 8,855                                 | United Gas Corp.....                                   | 144,892.59             | 266,757                        |
| 4,000                                 | United States Gypsum Co.....                           | 362,416.20             | 676,000                        |
| 4,750                                 | United States Plywood Corporation.....                 | 174,129.76             | 128,250                        |
| 6,800                                 | United States Steel Corporation.....                   | 299,635.01             | 333,200                        |
| 6,000                                 | Virginia Electric and Power Co.....                    | 130,447.67             | 188,250                        |
| 8,000                                 | West Virginia Pulp and Paper Co.....                   | 226,533.72             | 249,000                        |
| 1,700                                 | Weyerhaeuser Timber Company.....                       | 87,917.16              | 140,250                        |
| <u>495,572</u>                        | <i>Total Common Stocks.....</i>                        | <u>\$16,827,924.15</u> | <u>\$30,487,102</u>            |
| <u>528,722</u>                        | <i>COMMON AND PREFERRED STOCKS—Funds Invested ....</i> | <u>\$20,630,174.14</u> | <u>\$34,201,562</u>            |
|                                       | <i>AGGREGATE INVESTMENTS (BONDS AND STOCKS).....</i>   | <u>\$45,498,314.13</u> | <u>\$59,215,093</u>            |

# SCHEDULE OF SECURITIES—*Concluded*

## SUMMARY OF SECURITY TRANSACTIONS JULY 1, 1953 TO JUNE 30, 1954

Cash awaiting investment—July 1, 1953..... \$286,954.59

### *Sales and Redemptions*

|  | Gain         | Loss         | Book Value     |                |
|--|--------------|--------------|----------------|----------------|
| Bonds .....  | \$24,375.63  | .....        | \$4,498,348.61 |                |
| Preferred Stocks .....                               | .....        | \$20,825.98  | 270,882.16     |                |
| Common Stocks .....                                  | 806,609.75   | .....        | 1,605,492.10   |                |
| Sale of Stock Rights.....                            | 4,395.32     | .....        | .....          |                |
| Mortgages .....                                      | 178.85       | .....        | .....          |                |
|  | <hr/>        | <hr/>        | <hr/>          |                |
| Net Gain—To Exhibit E.....                           | \$835,559.55 | \$20,825.98  | \$6,374,722.87 | 7,189,456.44   |
|  | <hr/>        | <hr/>        | <hr/>          |                |
|  | \$835,559.55 | \$835,559.55 |                |                |
|  | <hr/>        | <hr/>        |                |                |
| Income applied to amortization of bond premiums..... |              |              |                | 23,632.00      |
| Market value of stock dividend.....                  |              |              |                | 10,510.50      |
|  |              |              |                | <hr/>          |
| Total .....  |              |              |                | \$7,510,553.53 |

### *Acquisitions*

|   |                |              |
|---|----------------|--------------|
| Bonds .....                                 | \$4,139,896.72 |              |
| Common Stocks .....                         | 3,313,507.35   | 7,453,404.07 |
|   | <hr/>          | <hr/>        |
| Cash awaiting investment—June 30, 1954..... |                | \$57,149.46  |
|   |                | <hr/>        |

SCHEDULE 3

SUMMARY OF CHANGES IN CURRENT FUNDS SURPLUS ACCOUNTS AND RESTRICTED GIFTS AND GRANTS

FOR THE YEAR ENDED JUNE 30, 1954

|   | ADDITIONS               |   |  |                  |   | DEDUCTIONS                   |                           | Balance<br>June 30, 1954 |
|---|-------------------------|---|--|------------------|---|------------------------------|---------------------------|--------------------------|
|   | Balance<br>July 1, 1953 | Trustees'<br>Appropriations<br>(Schedule 4) | Allotments<br>and<br>Transfers—<br>Net | Other<br>Credits | Transfer<br>From<br>Special<br>Funds<br>(Exhibit E) | Expenditures<br>(Schedule 4) | Revertments<br>(see Note) |                          |
| Current Funds Surplus:                    |                         |   |  |                  |   |                              |                           |                          |
| Appropriated:                             |                         |   |  |                  |   |                              |                           |                          |
| Departmental Research Operations:         |                         |   |  |                  |   |                              |                           |                          |
| Department of Plant Biology               | \$11,079.86             | \$106,250.00                                | \$1,175.00                             |                  |   | \$95,036.19                  | \$17,973.82               | \$5,494.85               |
| Department of Genetics                    | 9,520.35                | 177,535.00                                  | 3,500.00                               |                  | \$11,155.04   | 189,490.59                   | 8,659.94                  | 3,559.86                 |
| Dormitory and Mess Hall                   | 647.68                  | 1,200.00                                    |  | \$10,496.39      |   | 10,371.48                    |                           | 1,972.59                 |
| Geophysical Laboratory                    | 19,245.87               | 200,950.00                                  | 48,752.99                              |                  |   | 243,950.44                   |                           | 21,935.97                |
| Department of Archaeology                 | 5,238.50                | 103,300.00                                  |  |                  |   | 95,409.39                    | 3,062.45                  | 3,866.30                 |
| Mount Wilson Observatory                  | 13,697.69               | 283,150.00                                  |  |                  |   | 263,093.15                   | 18,291.14                 | 15,463.40                |
| Department of Terrestrial Magnetism       | 14,764.95               | 311,950.00                                  | 30,413.19                              |                  |   | 333,250.81                   |                           | 23,877.33                |
| Department of Embryology                  | 8,248.08                | 130,305.00                                  | 3,695.00                               |                  |   | 136,289.18                   | 2,969.09                  | 2,989.81                 |
| Total Departmental Research Operations    | \$82,442.98             | \$1,314,640.00                              | \$87,536.18                            | \$10,496.39      | \$11,155.04   | \$1,366,891.23               | \$60,219.25               | \$79,160.11              |
| Administration                            |                         |   |  |                  |   |                              |                           |                          |
| General Operations                        | 2,109.47                | 217,200.00                                  | 27,065.38                              |                  |   | 238,301.08                   | 2,088.36                  | 5,985.41                 |
| General Publications                      | 51,569.89               | 106,250.00                                  | — 100,070.23                           |                  |   |                              | — 611.00                  | 58,360.66                |
| Research Projects, Fellowships, etc.      | 63,437.77               | 30,000.00                                   |  | 5,785.38         |   | 34,190.22                    |                           | 65,032.93                |
| Pension Fund                              | 101,037.53              | 32,500.00                                   | 15,096.67                              | 3,946.35         |   | 91,579.89                    |                           | 61,000.66                |
|   | 147,933.51              | 145,000.00                                  | 5,872.00                               |                  |   | 158,050.52                   |                           | 140,754.99               |
| Total                                     | \$448,531.15            | \$1,845,590.00                              | \$35,500.00                            | \$20,228.12      | \$11,155.04   | \$1,889,012.94               | \$61,696.61               | \$410,294.76             |
| Unallotted:                               |                         |   |  |                  |   |                              |                           |                          |
| General Contingent Fund                   | 492,321.45              |   | — 35,500.00                            |                  |   |                              | — 61,696.61               | 518,518.06               |
| TOTAL CURRENT FUNDS SURPLUS—<br>EXHIBIT C | \$940,852.60            | \$1,845,590.00                              |  | \$20,228.12      | \$11,155.04   | \$1,889,012.94               |                           | \$928,812.82             |
| Restricted Gifts and Grants—Exhibit D     | 31,650.15               |   |  | 99,303.81        |   | 68,575.88                    |                           | 62,378.08                |
| TOTAL                                     | \$972,502.75            | \$1,845,590.00                              |  | \$119,531.93     | \$11,155.04   | \$1,957,588.82               |                           | \$991,190.90             |

NOTE: Reversions to:

GENERAL CONTINGENT FUND:

From Current Year's Appropriations—Schedule 4

From Prior Years' Appropriations

\$57,846.01

3,850.60

GENERAL OPERATIONS:

From Prior Years' Appropriations

\$61,696.61

611.00

Total

\$62,307.61



STATEMENT OF EXPENDITURES AND BUDGET SUMMARY INCLUDING MISCELLANEOUS RECEIPTS  
FOR THE YEAR ENDED JUNE 30, 1954

|   | EXPENDITURES AGAINST |             |                          |                   |   |
|---|----------------------|-------------|--------------------------|-------------------|---|
|   | Salaries             | Fellowships | Equipment<br>(Exhibit F) | Other<br>Expenses | <div>Current Year's<br/>Appropriations<br/>and<br/>Other Credits</div> <div>Prior<br/>Years'<br/>Appropriations</div> |
| Departmental Research Operations:   |                      |             |                          |                   |   |
| Department of Plant Biology.....  | \$74,472.97          | .....       | \$2,358.49               | \$18,204.73       | \$85,950.10   |
| Department of Genetics.....   | 135,696.56           | .....       | 6,434.50                 | 47,359.53         | 167,529.43  |
| Dormitory and Mess Hall.....  | 3,380.00             | .....       | .....                    | 6,991.48          | 9,723.80  |
| Geophysical Laboratory.....   | 166,146.26           | .....       | 25,126.00                | 52,678.18         | 211,395.93  |
| Department of Archaeology.....  | 69,193.65            | .....       | 325.17                   | 25,890.57         | 91,013.74   |
| Mount Wilson Observatory.....   | 209,678.34           | .....       | 4,052.50                 | 49,362.31         | 251,783.15  |
| Department of Terrestrial Magnetism.....  | 250,333.19           | .....       | 9,906.66                 | 73,010.96         | 309,174.46  |
| Department of Embryology.....   | 106,321.91           | .....       | 5,362.98                 | 24,604.29         | 127,946.75  |
| Total Departmental Research Operations.....   | \$1,015,222.88       | .....       | \$53,566.30              | \$298,102.05      | \$1,254,517.36  |
| Office of Administration.....   | 147,202.11           | .....       | 979.52                   | 90,119.45         | 228,787.12  |
| General Publications.....   | .....                | .....       | .....                    | 34,190.22         | 2,261.93  |
| Research Projects, Fellowships, etc.....  | 10,310.00            | \$52,620.74 | .....                    | 28,649.15         | 31,548.83   |
| Pension Fund.....   | .....                | .....       | .....                    | 158,050.52        | 145,000.00  |
| Total.....  | \$1,172,734.99       | \$52,620.74 | \$54,545.82              | \$609,111.39      | \$1,889,012.94  |
| Restricted Gifts and Grants (see contra income—Exhibit B).....                                  | 23,322.36            | .....       | .....                    | 45,253.52         | 68,575.88   |
| TOTAL.....  | \$1,196,057.35       | \$52,620.74 | \$54,545.82              | \$654,364.91      | \$1,957,588.82  |
| <i>Budget Summary</i>   |                      |             |                          |                   |   |
| Budgets approved by Trustees:   |                      |             |                          |                   |   |
| Appropriations July 1 to December 31, 1953.....   |                      |             |                          | \$913,315.00      |   |
| Appropriations January 1 to June 30, 1954.....  |                      |             |                          | 932,275.00        |   |
| Total Appropriations—Schedule 3.....  |                      |             |                          | \$1,845,590.00    |   |
| Other Credits—Exhibit B:  |                      |             |                          |                   |   |
| Sales of publications.....  |                      |             |                          | \$5,785.38        |   |
| Proceeds from Dormitory and Mess Hall, Cold Spring Harbor.....                                  |                      |             |                          | 10,496.39         |   |
| Miscellaneous.....  |                      |             |                          | 3,946.35          |   |
| Total.....  |                      |             |                          | 20,228.12         |   |
| Revertment of unexpended current appropriations to General Contingent Fund—Exhibit B.....       |                      |             |                          |                   | 57,846.01   |
| Reserved from this year's appropriations for current liabilities and commitments—Exhibit B..... |                      |             |                          |                   | 145,856.87  |
| TOTAL.....  |                      |             |                          | \$1,865,818.12    | \$1,865,818.12  |





REPORT OF THE PRESIDENT  
OF THE  
CARNEGIE INSTITUTION OF WASHINGTON

FOR THE YEAR ENDING JUNE 30, 1954

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# REPORT OF THE PRESIDENT OF THE CARNEGIE INSTITUTION OF WASHINGTON

In accordance with provisions of the By-Laws of the Institution, the President has the honor to report to the Board of Trustees

on the condition of the Institution, on the inauguration of its new Retirement Plan, and on its program of research.

## A NEW RETIREMENT PLAN

A new Retirement Plan was inaugurated on July 1, 1954. It required much effort to bring this plan into final form, for there was involved a considerable departure from conventional practice. A discussion of some of the features of the plan and the reasons for their adoption may hence be of value.

The new plan was, of course, fully described in bulletins and in communications to the staff; and the staff members agreed to the plan before the final steps were taken. It is therefore not necessary to review the plan in detail, but its primary features warrant further discussion.

There has been much recent interest in new retirement plans, which depart widely from the accepted ideas of a generation ago and usually provide for the inclusion of equities in the investment portfolio. This interest is hardly surprising in the light of the marked decrease in the purchasing power of the dollar since the war. The Institution felt the effect of this decrease seriously, for some eighty retired staff members or their widows were inconvenienced; some of them were confronted with real hardship. This occurred when the purchasing power of their retirement benefits, at best about half of salary at the time of retirement, was cut approximately in half by the depreciation in real value of the dollar. The effect was mitigated by special supplementary retirement benefits, on a temporary basis, voted by the Trustees from the operating funds of the Institution.

It certainly would seem that a retirement plan could be devised which would give

better protection than this. Yet adequate protection is not a simple thing to achieve, and mere investment in common stocks is by no means a complete answer. A great change in traditional thinking is here involved, as well as the whole concept of trusteeship with its complex legal restrictions. The practice over many generations, in banks, insurance companies, and philanthropic institutions, has embedded in our thinking and even in our language ideas and phrases which have acquired meaning in a conventional sense but which will hardly stand a critical examination. One such slogan is: Investment in government bonds is conservative and safe. This is recited in spite of the fact that money thus invested has lost one-third of its purchasing power in a decade. Another is: The full duty of a trustee is to preserve the dollars committed to his charge, with such increment as is consistent with this primary duty. This point of view is often forced by law, and quite rightly so under many circumstances, as the only clearly specifiable safeguard to protect the public. But it is not always consistent with the broader duty of a trustee to act to the best advantage of those dependent upon him. A balance sheet which shows full maintenance of assets in dollars is often regarded as sound, even when its showing in terms of real value is appalling; and the person who suggests that mass savings held by institutions might be employed directly in industry with full participation in its successes and failures is sometimes spoken of as a mildly dangerous radical.



But the other side of the coin is also sometimes misrepresented. Common stocks are not a panacea for all ills. There is danger of loss in this area also, as history, even recent history, clearly shows. With a well diversified portfolio of investments in substantial companies, the effect of loss on individual investments may not be serious. With an adequate portion of the total investment in dollar securities, the dangers of a depression may be viewed with some measure of equanimity, and it would appear that such depressions as have occurred in our time could have been weathered. On the other hand, a far more severe depression might bring down the entire investment structure, not just a part of it, leaving disorder throughout the whole economy. The greater danger resides in possible action by governments. For they can do strange things, and do. They not only can devalue currency and thus in effect seize savings dependent upon their bonds; they can also seize control of industries, as a part of a scheme of socialization, creeping or abrupt, and do so by a process of depressing values and then compensating for them in an arbitrary manner.

The fact is that there is no such thing as absolute security. There is no investment without risk. No system, no formula, can guarantee that individual savings will provide at some future date an assured standard of living. The best that can be done is to balance risks in accordance with the judgment of wise and seasoned men, using history as a guide and modifying the lessons of history by sober estimates of current trends and future prospects.

If one could depend upon history's repeating itself, the problems would be relatively simple. For the financial history of the past eighty years contains clear lessons. Over that time the average income from common stocks, including appreciation in market value as well as dividends, has been roughly twice that from fixed income-bearing securities. A retirement plan based entirely on equities, supported by contribu-

tions of reasonable amounts in relation to salaries, would have provided a real post-retirement income adequate for a dignified standard of living if carried on over thirty-year periods of those eighty years. A plan based entirely on dollars would not have done so. A retirement plan based half on equities and half on bonds would have come closer to providing an invariant real income after retirement than one based on bonds alone. The great depressions would not have been fearsome in reality; for, although the dollar income of the benefit recipients would have diminished during such periods, the cost of living dropped as well, and the purchasing power of the part of the retirement benefit based on equities would still have held its own. These considerations point to extremely interesting possibilities even when the judgment of financial men regarding the relative merits of individual common stocks is eliminated, for example, by following the history of all stocks listed on the exchange at the date of the inauguration of a plan. Presumably the yield could be bettered by the exercise of judgment, but the lessons are clear even without the presence of judgment. History is the best guide we have, but we cannot be sure it will repeat itself, even in its major features. To accept it absolutely would be naïve, but to disregard it would be shortsighted indeed.

A well devised retirement plan, including substantial investment in equities, will ride through a depression safely, if such a depression is not more severe than those of our past experience; and benefit recipients will be subjected to a fluctuation of income but not real distress during the period, and will again prosper when it is over. How then about the trustees themselves? What will be their position if the market value of their equity portfolio tumbles to a fraction of its dollar value in a year, or even tumbles well below its original dollar cost? Will they be regarded by the beneficiaries of the trust as having properly discharged their responsibilities as

trustees? This is, of course, the heart of the problem and can by no means be brushed aside. It is the primary reason why trustees, even when not constrained by law, have preferred to rest their case upon the preservation of funds in dollars, perhaps while realizing that this restriction shuts the door to a greater over-all benefit for those dependent upon them. It is a sound reason why trustees should be conservative in terms of dollars, even though they may not be conservative in regard to the balance of risks. It is a sound reason why safe investments only, safe in terms of dollars, should be used by trustees when there is no other way in which unfair criticism can be avoided, especially with respect to great public institutions handling public savings in large amounts, which must be safeguarded against the charge, or the actual presence, of capriciousness or conflict of interest in the selection of securities.

But we are becoming more mature in this country on financial matters; and a group of professional scientists, skilled among other things in mathematical relationships, is not the general public. If an institution comes to a full understanding with such a group at the outset, expressing intentions and methods in full, spreading out in detail every step in accounting and the like, securing advance individual assent to the plan based on this full presentation, then there is little risk of widespread unjust criticism, whatever happens. With such a full understanding it is possible to exercise judgment and to aim for the benefits to be derived, on a long-range basis, from well considered equity investment. This is the basis on which the Institution's new plan has been started. No similar plan should be entered upon except under equally favorable circumstances.

There are a number of other principles that should be kept in mind in any plan of this sort. Investment in common stocks and realization thereon should both be spread over a period of years. When this

is done, one will expect, if history repeats itself, to approach the average benefits which history presents. Departure from the principle invites fluctuations and disparity between the benefits of individual participants. The ratio between classes of securities in the portfolio should be reviewed frequently by a body of men of seasoned financial judgment, not only because the ratio of yields from classes of securities varies greatly over the years, but also because the estimate of relative risk and of relative future yield cannot be made once for all. Any self-contained retirement plan of this sort should have a sufficient number of participants to ensure that probable departures from general experience will not involve severe burdens. The requisite size of the group is readily determined by the theory of samples. There are excellent papers on the subject, and the minimum advisable size of the group is not large.

With a large proportion of equities in the portfolio there can be no guaranteed income in dollars, although, of course, that portion of the fund which is invested in high-grade bonds will have assurance of the retention of dollar values. The guarantee, and the only guarantee, under the new plan is that the sum total of the contributions made by individuals or on their behalf, together with their accumulations of income before and after retirement, subject to any appreciation or depreciation in market value, will be returned in its entirety to the participating individuals in the form of post-retirement income, with the total amount received by one individual depending, of course, upon his longevity. Options for dependents may of course be introduced, provided they do not substantially alter the relationship between individual participants. The funds gained through the early demise of some participants are expected to offset the funds needed to carry others who live long.

The absence of any guarantee of retirement benefits in terms of dollars gives op-



portunity for great flexibility in the management of the funds. For one thing, it obviates the need to accumulate reserves. There need be no artificiality about the mortality table that is adopted; it may represent as close an estimate as possible of life expectancy, and need not be shaded for safety's sake to build reserves. The system, moreover, may be made to approach self balance; and in the long run, faults of the mortality table may be made self correcting. Each year the assets and liabilities of the retirement fund can be compared, and adjustments can be made in retirement benefits to keep them in balance. When this is done, the full benefit of the plan can be passed on to the retired members of the staff, thus increasing their retirement benefits above those that would be possible if reserves were being built for possible future needs, while retaining the essential element of all retirement and insurance plans, that is, the sharing of risks due to unpredictable individual longevity.

There are many details to be considered when new plans are set up. The tax aspects need careful examination, and review by the Internal Revenue Service is essential for final assurance in this regard. The

methods of computing and accounting need to be specified in full detail. This offers some difficulty, not because the mathematics is abstruse (in fact it does not extend far beyond arithmetic), but because it is difficult to pin down definitions precisely. The language of actuaries, like that of all specialists, tends to become obscure to laymen. But all these matters can and should be made precise.

The Institution has paid close attention to all these points and many more. Several of the Trustees have devoted much time and effort to the subject, and we have also had sound guidance from men with intimate personal experience in the subject.

The new retirement plan is in effect. We trust it will provide for a generation to come, not full protection to the staff against the hazards that accompany saving for the future (for no plan can accomplish that), but a sound basis on which the Retirement Fund can be managed intelligently to secure as much comfort in old age as possible and a balanced provision against the risks that all savings inevitably face. We also hope that our efforts in working this matter out in detail may be of some service to others.

#### RESEARCH ACTIVITIES

Fully one-half of the observing time of the 60-inch, 100-inch, and 200-inch telescopes at the Mount Wilson and Palomar Observatories has been regularly devoted for many years to spectroscopic and photometric studies of individual stars. These studies were planned to yield information on chemical compositions, temperatures, masses, densities, magnetic fields, luminosities, and the relation of luminosities to other factors. The studies, combined with similar investigations at many other observatories, extending over the past half century, have built up an extensive mass of information concerning the brighter stars, all of which are located in the neighborhood of the sun. From the results obtained it has been possible for the theorists

to construct models of the interior structure of various stellar types and thus to account quantitatively for the enormous energy radiated by the stars in terms of thermonuclear reactions in their very hot cores. From these models in turn it has been possible to construct an evolutionary history of the stars.

The spectroscopic and photometric study of stars was given an entirely new direction during World War II when Walter Baade, using the 100-inch telescope, investigated extensively the structures and stellar content of the great spiral galaxy in Andromeda, which is closely similar to our own Milky Way system. Baade's statistical studies of the relatively few stars bright enough to be observed with the 100-inch

telescope showed that the stars near the nucleus of this galaxy have a relation between color and luminosity that differs greatly from that of the stars in the spiral arms and of the stars in the neighborhood of the sun, which is located far out in one of the spiral arms of the Milky Way. This new kind of stellar population, located in the nuclear region, was designated as type II to differentiate it from the better-known type I, which is characteristic of the spiral arms and therefore of the stars near the sun.

It at once became evident that the type I stars, to which all the earlier detailed study had been given, could no longer be considered typical of all stars. Indeed, present evidence leads us to believe that there are many more stars of population II in the universe than there are of population I. Obviously it is important to obtain information about the properties of population II stars, similar to that already accumulated for the bright population I stars in the solar neighborhood.

The nearest large samples of pure population II stars are found in globular clusters so distant that the individual stars are many thousands of times fainter than the corresponding near-by stars that were used in the earlier study of population I. It was fortunate that the Hale telescope became available shortly after Baade's discovery, for without its great light-gathering power and the efficiency of its photometers and spectrographs it would have been impracticable to investigate effectively the properties of the stars in these clusters.

Extensive spectroscopic investigations of the globular-cluster stars have been carried out by Armin J. Deutsch, Jesse L. Greenstein, Guido Münch, and Olin C. Wilson during the past two years. Preliminary results indicate a lower excitation temperature for a given spectral class and a substantially lower abundance of the metals than is found in population I. Photometric studies by Halton C. Arp, William A. Baum, Donald E. Osterbrock, Allan R.

Sandage, and Merle F. Walker have revealed large differences between the two populations in the relation between total luminosity and surface temperature as indicated by color measurements. These early results suggest that the two stellar types have evolved differently, the population II stars being in general much older than those of population I. Both in the Milky Way and in the Andromeda galaxy, one of the outstanding differences between the nuclear region and the outer region in which the spiral arms are located is that the outer region contains many huge clouds of dust and gas. It is now believed that many of the population I stars were condensed from these clouds late in the history of the galaxies, whereas the population II stars are nearly as old as the galaxies themselves.

An important, new co-operative project of the Mount Wilson and Palomar Observatories and the Department of Terrestrial Magnetism of the Institution, the National Bureau of Standards, the U. S. Naval Observatory, and the California Institute of Technology, aided by funds from the Carnegie Corporation of New York, is aimed at examining carefully whether electronic image-conversion techniques offer a means for extending the range of large telescopes or of increasing the utility of those of moderate size. The photographic method employed by the large telescopes is limited by the inability of photographic emulsions to store useful information beyond a point which is fixed for a given emulsion by the ever present glow of the night sky and by the focal ratio of the telescope. The program will undoubtedly be of several years' duration.

At the Department of Terrestrial Magnetism considerable progress in our understanding of the structure of the atomic nucleus has been made on the basis of a new observation, namely, that most nuclei from fluorine through uranium can be



made to radiate characteristic gamma-ray lines when bombarded by relatively low-speed helium ions from an electrostatic generator. No direct nuclear encounter takes place; the nucleus is merely swept into one of its excited quantum states by the electric field of the passing particle, after which it returns to its ground state with the emission of a gamma ray. The energy of this gamma ray can be measured accurately with modern scintillation crystals and counting techniques. This measurement locates the position of energy levels in the nucleus, much as the study of the line spectra of elements excited in the electric arc reveals the locations of electronic energy levels in the outer atom.

About 150 energy states in some 70 nuclei examined at the Department have been detected by this "Coulomb excitation" process. About half of these were previously unknown, and some are not accessible by any other means. The majority of them can be identified with theoretical predictions for a special type of rotational motion of nonspherical nuclei. Investigations of this kind are based on a recent nuclear model that endeavors to reconcile the liquid-drop and shell-structure aspects of nuclear matter. This is the so-called collective model of the nucleus, proposed by Aage Bohr and B. R. Mottelson in Copenhagen three years ago. It is remarkable that this model predicts the position, angular momentum, and lifetime of nuclear energy levels for a large number of the nuclei studied this year by Coulomb excitation at the Department.

The Department's analysis of the data available from continuous registration of cosmic-ray ionization at four stations since 1937 has now revealed a variation of cosmic-ray intensity, which is similar at all stations, well correlated with sunspot numbers. The range of this variation is nearly four per cent, with its maximum near sunspot minimum. The Department had found some years ago that decreases of cosmic-ray intensity occur during some mag-

netic storms, but these effects alone are too infrequent and too small to account for the variation which has now been demonstrated. Hence this variation requires the more or less continuous operation of a mechanism, not yet understood, which may also be responsible for an eleven-year variation in the earth's magnetic field. In recent years there has been vigorous theoretical discussion in the scientific community concerning the possibility that so-called cosmic rays may be localized or perhaps even trapped in orbits in our solar system. These two observations have a bearing on theories of this type, which involve the assumption of extensive magnetic fields in large regions of space.

The study of the crust of the earth using seismic waves from large explosions led to a new understanding of an old puzzle this year. The puzzle has been to account for the continuous agitation of seismic instruments for a long period after the arrival of the first shock, not only in oil-prospecting studies covering distances of a few thousand feet, but also in earthquake observations at great distances. One might expect several sharp impulsive movements, corresponding to wave fronts arriving by each of several paths, but instead of this the ground moves vigorously and continuously after the first impulse arrives.

The explanation, developed out of several years of analysis of field tests and recent model experiments using micro-second impulses in the laboratory, is simple indeed. It turns out that the impulsive wave from the source in passing over the hills and valleys of the earth's surface causes each of these structures to send out secondary wave motions; and many of these secondary waves are of a type confined to propagation along the surface with a slower speed than that of the primary impulse and with low attenuation. Thus a receiving instrument, after the first impulse, is immersed in a whole interference pattern of surface wave motions which obscure its response to buried structures. Fortunately,

it is still possible to obtain significant information about the earth's interior by using many careful measurements. In addition, since the nature of the extensive earth motion induced by an explosion is now better understood, there is less likelihood of erroneously ascribing these interference patterns to internal earth structures. The results obtained now rest on a better understanding of the entire problem of wave motions in relation to earth structures.

Experimental observations at the Geophysical Laboratory are gradually providing a quantitative basis for understanding the various assemblages of minerals found in the metamorphic rocks. These rocks, which are one of the three major types, are in many cases formed from sediments as a result of high temperatures and pressures.

The vast amount of material that passes through the weathering cycle is ultimately deposited on the earth's surface in areas of low elevation. Large parts of these areas are not only low but actually subsiding. Materials deposited in this fashion are thus brought to progressively greater depths in the crust, depths characterized by temperatures and pressures far higher than those at the surface. In this new environment the sediments are entirely reconstituted. During the initial stages of their metamorphism, mineral assemblages stable at moderate temperatures and pressures are formed. These subsequently give way to assemblages that form, or are able to survive for long periods, at temperatures and pressures characteristic of the deeper levels of the crust of the earth. Generations of field geologists have managed to establish a series of mineral assemblages (facies) that are thought to characterize different pressure and temperature environments in the crust. Thus, assemblages containing chlorite are ordinarily regarded as being of "lower grade" than those containing biotite or garnet.

Although geologists agree in most respects about the detailed interpretation of

differences in facies, they are in almost total disagreement about the theoretical basis of metamorphic action. This subject has been examined by a number of workers in recent years, and it now seems certain that the serious theoretical differences that have emerged from the discussions can be resolved only through careful and extensive experimentation. Such work at the Laboratory has led to the synthesis of many of the minerals found in metamorphic rocks.

A series of micas and garnets have been synthesized by Hatten S. Yoder, Jr., and Hans P. Eugster, some for the first time, and their ranges of stability as functions of temperature and pressure have been determined. These are the dominant minerals of the common schists and are key minerals in establishing the grade of metamorphism. The two principal micas, muscovite and phlogopite, have been studied; and the investigation has been extended to include micas at first believed to be of minor importance. After the precise properties of these micas were established through synthesis, it became apparent on re-examination of the rocks that they occurred in much greater abundance than had been supposed. The garnets, principally almandite and pyrope, are among the most striking minerals in a metamorphic rock, mainly because they usually exhibit regular forms in the presence of other growing crystals. The stability curve of almandite garnet has been measured, and insight has been gained into its occurrence in metamorphic rocks as well as in some igneous rocks. The amphiboles, which are of paramount importance in the amphibolites and garnet amphibolites, have yielded to synthesis. Tremolite, the first mineral to form in the metamorphism of a siliceous dolomite, was investigated and its stability range outlined. Of course, the synthesis of these minerals, itself a major advance, is only the beginning. It is now possible to study the interrelation of these minerals and establish the exact limits of stability of



the assemblages. This is the key to understanding the metamorphic processes.

Studies of the morphology of fossils have provided the basis for much of our present understanding of structural geology and of the evolution of life. Work at the Laboratory by Philip H. Abelson has now shown that further information about these subjects can be found in the chemical constituents of the fossils, many of which contain organic compounds such as amino acids. The studies have demonstrated that the occurrence of proteins in hard parts of recent creatures is widespread and that under favorable conditions of preservation fossils may contain amino acids after as long as 360 million years. Among the compounds found in ancient specimens were alanine, glycine, valine, leucine, aspartic acid, and glutamic acid. These are identical with some of the building blocks utilized in present-day proteins.

The thermal stabilities of some of the amino acids were measured for short periods of time at high temperatures to indicate the rates and nature of degradations that might have occurred during geological periods at lower temperatures. From these studies it was possible to predict successfully which amino acids would be preserved in the fossils. It was noted that the rate of degradation of the amino acids is sharply increased with increasing temperature. These facts suggest the possibility of a recording geological thermometer for sediments. The discovery of amino acids in fossils also opens up a new opportunity for investigating the biochemistry of creatures long extinct.

At the Department of Plant Biology L. N. M. Duysens has continued his investigations of the changes in the absorption spectrum of purple bacteria caused by illumination. The nature of these changes suggests that a cytochrome-like enzyme participates in the photosynthesis of these bacteria. Spectral changes of unidentified substances have been found in illuminated

*Chlorella* suspensions. This new method of investigating the process of photosynthesis provides a direct way to measure the participation of some of the previously unknown components of the plant's photosynthetic system.

About thirty of the range-grass strains developed by the Department are now producing seed at the Pullman, Washington nursery of the U. S. Soil Conservation Service, in row plantings ranging from 60 to 600 feet in length; and in California four additional strains are being grown in approximately half-acre lots for seed increase. The seed harvest will be used for field testing of the new bluegrass hybrids in various climates and under different schedules of management. The grass work this year has led to the interesting discovery that environment may have great influence in altering the percentage of aberrant or "off-type" plants that may appear in a stabilized, asexually reproduced strain. Under conditions of intense competition in unfavorable environments, the percentage of sexually produced variants may increase greatly in relation to the normally predominant asexual plants, which usually crowd out the variants. This finding is of considerable importance in understanding natural selection in wild species, and explains the lack of uniformity sometimes observed in commercially produced seed lots of established strains.

The absolute absorption spectra of purified chlorophylls *c* and *d* and of bacteriochlorophyll have been determined by James H. C. Smith and Allen Benitez. This provides a means for the quantitative analysis of these pigments in plants which use them for photosynthesis instead of chlorophylls *a* and *b*, used by ordinary higher plants.

Studies of forests on both sides of the Pacific continue to yield information regarding the history of plants. The coast redwood appears not to have been a tree of the coast during much of its hundred-million-year history, but to have lived at

higher elevations in the interior of North America. In Asia, where the original vegetation cover has been largely destroyed by man, there are still significant clues to the relationships of fossil floras of the past. With field work in China no longer possible, the forests of Japan provide the most nearly adequate solution to many of the problems of Tertiary paleobotany.

Additional information about the structure of genes has been obtained at the Department of Genetics by M. Demerec and his collaborators, working with the bacterial species *Salmonella typhimurium*. In every one of fifteen cases tested so far, their experiments have indicated that a gene locus occupies a longitudinal section of the chromosome, and that changes occurring in different parts of the locus give rise to different new forms of the gene, that is, alleles. Their data show, furthermore, that crossing over—the exchange of segments between the homologous parts of two chromosomes—can take place very readily within the gene locus. By studying crossing over involving several alleles of adjacent genes, they have been able to determine the sequence of regions within a gene locus where the changes responsible for certain alleles have occurred, and to make an approximate estimate of the distances between these regions.

Alfred D. Hershey in his earlier work showed that the genetic mechanism of bacterial viruses (bacteriophages) is very similar to that which operates in higher organisms. In viruses it is possible to carry out at least a partial chemical analysis of this mechanism. Two lines of evidence recently developed by Hershey indicate that viral nucleic acid controls viral inheritance. The first of these depends on the fact that nucleic acid transferred from parental to offspring phage (analyzed radiochemically) has a characteristic composition that cannot be altered by competitive substrates added to the culture medium. The second line of evidence

emerges from an analysis of the kinetics of protein and nucleic acid synthesis in infected cells, which seems to show that viral nucleic acid is formed before, and so presumably determines, viral protein.

The studies of cell chemistry by Berwind P. Kaufmann and Margaret R. McDonald have focused attention on the importance of the nucleoprotein complex in vital cellular activities. "Dissection" of the living cell with enzymes has shown that the ribonucleoproteins, as well as the deoxyribonucleoproteins, are essential for the maintenance of normal chromosome form and distribution.

At the Department of Embryology, Robert K. Burns has made notable progress in the analysis of the control of sex differentiation in the embryo by action of sex-gland hormones. Using the opossum, in which embryonic animals are accessible in the mother's brood pouch, Burns some years ago succeeded in reversing the sex character of the accessory reproductive organs, from female to male and vice versa, by the injection of male and female hormones respectively. In a few experiments he obtained evidence of reversal even of the primary sex organs, the testes of male opossums showing the beginnings of change into ovaries. Improving the timing and dosage of the injections by following up clues obtained from his earlier observations, Burns in his experiments in Florida in February and March 1954 carried the reversal much farther, causing the embryonic male opossums to develop unmistakable if somewhat atypical ovaries in which ovocytes (egg cells) were found in primordial ovarian follicles. This is the first report of sex reversal of the mammalian gonad by deliberately planned experimental means, and the success obtained is of great importance for the study of sex differentiation in higher animals.

A long-term study of the blood flow through the placenta, carried out by Elizabeth M. Ramsey, first in monkeys bred for



the purpose at the Department of Embryology's monkey colony, and afterward on human placentas supplied by physicians, reached significant conclusions. Maternal blood appears to get into the placenta by arteries entering at its base, and to be drained away by veins which also open at the base of the placenta as well as through a venous sinus at the placental margin. The problem, briefly, is to discover why then the blood does not flow directly between these adjacent vessels, short-circuiting the upper levels of the placenta, where it is needed to exchange vital materials with the infant's blood flowing through the fetal vessels in that region. Various ingenious hypothetical explanations which have been proposed would require the course of the maternal vessels to be different from that stated above. Ramsey's studies, on the other hand, confirm in general the relatively ample basal drainage, but point to physiological factors, that is to say differences in blood pressure between the maternal arterial and venous blood and the blood within the placenta, as the chief directive agency of the maternal placental circulation, assisted by various anatomical features of placental structure.

In order to avail himself of the Department's special experience in primate research and facilities for providing pregnant monkeys, A. St. G. Huggett of St. Mary's Hospital Medical School, London, spent the year at the Department of Embryology. His special aim was to study the transmission of sugars from mother to fetus, and in particular to find out whether the primate placenta converts glucose into fructose as is the case in the ungulate (sheep) placenta. For this work a team of experienced investigators and technicians led by S. R. M. Reynolds was organized. Twelve pregnant rhesus monkeys were provided. It appears that sugar metabolism in the primate placenta is not like that in sheep, at least with respect to glucose and fructose. The question is of importance not only in general metabolic physiology,

but also in the study of diabetes and other disturbances of carbohydrate metabolism in pregnancy.

Two specimens added during the year to the great collection of sectioned primate embryos have a particularly interesting history, which reflects the international character of scientific research. These are two embryos of the queer little East Indian animal *Tarsius spectrum*, which is of great importance in the evolutionary history of the apes, monkeys, and man. Collected on the island of Banka in 1894-1895 and preserved in alcohol for the Hubrecht Laboratory of Utrecht, Holland, they were later presented to Hans Bluntschli of Bern, Switzerland, and came to Baltimore in 1950 when Bluntschli's collection was acquired by the Department of Embryology. Thus they have traveled almost around the world and have resided at three of the great centers of embryological research; and finally, with their whole long history attested by complete records, they have been made ready for microscopic study, taking their place along with the embryos of a dozen other species of primates in the Baltimore laboratory. When the bottles were opened the tiny embryos were found to be in perfect condition. Under the microscope their cell structure is as well defined as if they had been prepared this year, instead of sixty years ago.

The Department of Archaeology has continued its program of researches dealing with the preconquest history of Yucatan. An important discovery resulting from excavation at the ruins of Mayapan came with the unearthing of a mural painting in one room of a large temple building. Although numerous fragments of fallen wall plaster with painted designs had previously indicated that this form of art was by no means uncommon at Mayapan, this is the first example sufficiently well preserved to reveal the style of the painting or the subject of the composition. The scene shows five buildings resting on a

common platform or terrace. The wall of the terrace is decorated with the heads of monsters painted in green, red, yellow, white, and blue against a black background. Much of the upper part of the mural is gone; but enough remains to reconstruct the outlines of the buildings, probably temples, and to see that they were painted with designs in polychrome. The architectural style portrayed suggests a mixture of Maya and Mexican traditions and is of a sort unexampled in any other known remains of buildings in Yucatan.

One important aspect of the work in Yucatan is the testing, by means of archaeological procedures, of the all too brief and frequently unreliable documentary records concerning the preconquest history of the country. Interesting information came to

light this season relating to the supposed abandonment of Mayapan, the last great city of the Maya. This important event is referred to in the chronicles as the "abandonment," "depopulation," or "destruction" of the city and is associated with fighting, seemingly civil strife. For the most part these historical references are so vague that they leave one wondering as to the time and nature of the event, and whether the town did indeed come to an abrupt end. The finding this year of rather clear evidence of the sacking and burning of several buildings goes far toward confirming the events described by the old records, and parallels in many ways the archaeological verification of the Homeric account of the destruction of Troy.

#### STAFF

Among the honors that have come to Institution staff members during the report year, one of unusual human interest was the election of Horace W. Babcock, staff member of the Mount Wilson and Palomar Observatories, to the National Academy of Sciences on April 27, 1954. Babcock's father, Harold D. Babcock, who is a retired staff member of the Observatories, has been a member of the Academy since 1933. It is a rare circumstance for father and son to be members at the same time. Both the elder and the younger Babcock have done distinguished work in their investigations on the magnetic field of the sun.

The Gold Medal of the Royal Astronomical Society was awarded to Walter Baade, staff member of the Observatories, "for his observational work on galactic and extragalactic objects." Baade's recalibration of cepheid variable stars in 1952 indicates that the universe is about twice as large as had previously been supposed. Baade was also elected a foreign member of the Royal Astronomical Society of Canada. Olin C. Wilson, astronomer at the Observatories,

was elected President of the Astronomical Society of the Pacific.

At the Department of Terrestrial Magnetism, Ernest H. Vestine, a staff member, was elected to membership in the National Academy of Sciences on April 27, 1954.

M. Demerec, Director of the Department of Genetics, was elected President of the American Society of Naturalists for 1954. Oscar Riddle, retired staff member of the Department, has been made a Fellow of the Royal Society of Arts, London.

At the Geophysical Laboratory, J. Frank Schairer, physical chemist, received the Day Medal at the meeting of the Geological Society of America in Toronto, Canada, on November 9, 1953, for outstanding contributions in the applications of physics and chemistry to geology. The Academy of Natural Sciences of Philadelphia gave Norman L. Bowen, retired staff member of the Laboratory, the 1953 Hayden Memorial Geological Award, a gold medal, on November 12, 1953. Bowen was cited as "a leader in physico-chemical research in petrology, introducing systematic quantitative, experimental work on problems of



origins of minerals and igneous rocks." On March 25, 1954, he was elected an Honorary Member of the Mineralogical Society of Great Britain and Ireland.

Richard D. Grill, photographer at the Department of Embryology, received an award of merit for work exhibited at the 23d Annual Meeting of the Biological Photographic Society, Los Angeles, September 1953. He was also awarded a blue ribbon for pictures of human embryos shown at the 9th Chicago International Exhibition of Nature Photography, Chicago Natural History Museum, February 1954.

For his outstanding contributions to Southwestern and to Middle American archaeology, Earl H. Morris, staff member of the Department of Archaeology, received the Alfred Vincent Kidder Award of the American Anthropological Association for 1953.

The deaths of Dr. Herman A. Spoehr and Dr. Leo F. Stock brought a profound sense of personal loss to their many friends both inside and outside the Institution.

Dr. Spoehr, who retired in 1950 as Director of the Department of Plant Biology, died on June 21, 1954, after a short illness, in Palo Alto, California, at the age of sixty-nine. He was appointed a staff member of the Institution's Desert Laboratory at Tucson, Arizona, in 1910, moving in 1920 to the Coastal Laboratory at Carmel. In 1928 he became Chairman of the newly created Division (now Department) of Plant Biology, which moved to the Stanford University campus in 1929. After a year (1930-1931) as Director of the Natural Sciences Division of the Rockefeller Foundation in New York, Dr. Spoehr re-

turned to Stanford in 1931, resuming the chairmanship of the Division.

A gifted and versatile investigator of the first order, Dr. Spoehr's pioneer work on photosynthesis in plants led to the present widespread interest in the possibility of the large-scale culture of algae for food production. His book *Photosynthesis*, issued in 1926, is used by biologists and biochemists throughout the world. He also wrote a large number of authoritative technical papers dealing with the chemistry of carbohydrates and plant physiology.

Dr. Spoehr was a stimulating and inspiring director of research—learned, disciplined, patient, and friendly. In all his dealings his understanding and loyalty were a source of unfailing encouragement to his scientific colleagues.

Dr. Leo F. Stock, retired staff member of the Division of Historical Research, died suddenly on March 8, 1954, at the age of seventy-five. He joined the Division in 1910 and devoted a good share of his time to the editing of five volumes of the *Proceedings and Debates of the British Parliaments Respecting North America*, which were published by the Institution from 1924 to 1941. They constituted one of the essential parts of J. Franklin Jameson's plan to document the history of our country and are a necessary tool of the historian concerned with our colonial history. Their careful editing won the esteem of scholars and added much to the usefulness of the debates themselves. After his retirement in 1945 Dr. Stock collaborated with Dr. Elizabeth Donnan on a volume of the Jameson letters, *An Historian Looks at His World: Selections from the Correspondence of J. Franklin Jameson*, which will be published in 1955 by the American Philosophical Society.

#### FINANCES

Net income from investments, exclusive of stock dividends, during the fiscal year ended June 30, 1954 amounted to \$1,966,318.25. This is an increase of \$49,030.15

over the previous year. Appropriations authorized during the fiscal year by the Board of Trustees amounted to \$1,845,590.00; and additional funds made avail-

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able by the Executive Committee from the General Contingent Fund during the year amounted to \$35,500.00, bringing the total amount of funds authorized during the year to \$1,881,090.00, with the result that net investment income exceeded total appropriations and allotments by the Executive Committee by \$85,228.25. This excess of income over appropriations and allotments was added to reserves during the year.

The yield at book value of securities held during the year was 4.35 per cent, and the yield at the market value of the portfolio on June 30, 1954 was 3.43 per cent.

The proportion of the Institution's portfolio invested in common stocks at the close of the year, at market value, was higher than a year ago; this was due largely to the steady rise of the stock market during the greater part of the year. Common stocks held on June 30, 1954 (at market value) represented 51.4 per cent of the portfolio, bonds 42.2 per cent, preferred stocks 6.3 per cent, and cash 0.1 per cent, as compared with 44.3 per cent in common stocks, 47.8 per cent in bonds, 7.3 per cent in preferred stocks, and 0.6 per cent in cash a year ago. The book value of securities held at the close of the year was \$45,498,314.13, an increase of \$1,055,049.20 over the

previous year; and the market value of the securities was \$59,215,093.00 as compared with \$50,224,304.00 a year ago.

The Institution's income from common stocks accounted for 58.8 per cent of its total investment income; income from bonds accounted for 33.2 per cent, and income from preferred stocks accounted for 8.0 per cent, an increase of 2.8 per cent in the proportion of investment income received from bonds and a corresponding decrease in the proportion from common and preferred stocks as compared with the percentages of the previous year.

The Institution has continued to support a vigorous scientific program at only a slight increase in cost over the previous year, and a modest amount has been added to reserves which have accumulated over the past years. Since a large part of the Institution's income results from investments in common stocks, these reserves provide a cushion against a possible future reduction of income from equity holdings.

Statements showing the financial position of the Institution on June 30, 1954, together with a certificate of Haskins & Sells, Certified Public Accountants, appear with the report of the Executive Committee.

VANNEVAR BUSH





REPORTS OF DEPARTMENTAL ACTIVITIES  
AND CO-OPERATIVE STUDIES

ASTRONOMY

*Mount Wilson and Palomar Observatories*

COMMITTEE ON  
ELECTRONIC IMAGE CONVERTERS FOR TELESCOPES

TERRESTRIAL SCIENCES

*Department of Terrestrial Magnetism  
Geophysical Laboratory*

BIOLOGICAL SCIENCES

*Department of Plant Biology  
Department of Embryology  
Department of Genetics*

ARCHAEOLOGY

*Department of Archaeology*



# MOUNT WILSON AND PALOMAR OBSERVATORIES

OPERATED BY THE CARNEGIE INSTITUTION OF WASHINGTON  
AND THE CALIFORNIA INSTITUTE OF TECHNOLOGY

*Pasadena, California*

IRA S. BOWEN, *Director*

## OBSERVATORY COMMITTEE

IRA S. BOWEN, *Chairman*  
WALTER BAADE  
HORACE W. BABCOCK

ROBERT F. BACHER  
JESSE L. GREENSTEIN  
ERNEST C. WATSON

Almost immediately after the formation of the Carnegie Institution of Washington in 1902, advisory committees were appointed for the several sciences to make recommendations as to how the Institution's resources could be most effectively used to advance their respective fields of investigation. The members of the committee for astronomy were E. C. Pickering, Lewis Boss, G. E. Hale, S. P. Langley, and Simon Newcomb. This group made two major recommendations, namely, that an observatory be established in the southern hemisphere, and that a station for observing the sun be located in a region of this country having the finest possible climatic and seeing conditions. A smaller committee consisting of Hale, Boss, and W. W. Campbell was then asked to investigate these proposals and make detailed recommendations.

As a first step in the investigation of the second recommendation, W. J. Hussey was employed to make a study of climatic and seeing conditions in southern California and Arizona during the summer of 1903. It is of interest to note that the two locations where he found conditions to be most nearly ideal for astronomical observations were Mount Wilson and Palomar Mountain. In his report, however, the latter site was not recommended because of its inaccessibility at that time. On the basis of these studies Hale visited Mount Wilson several times in late 1903 and early 1904. Finally, Hale brought an expedition from Yerkes Observatory and established a temporary station on the mountain on Febru-

ary 29, 1904, to continue the investigation of observing conditions. During the summer of 1904 a grant of \$10,000 from the Institution made it possible to ship the Snow solar telescope from Yerkes Observatory and to reconstruct it on Mount Wilson. The observing conditions found during the operation of this temporary station were so satisfactory that at the annual meeting of the Trustees of the Institution in December 1904 an appropriation of \$150,000 was made for the establishment and operation during the ensuing year of a solar observatory on Mount Wilson as a department of the Institution, with Hale as its Director.

The present year, therefore, marks the end of the first half century of operation of the Mount Wilson Observatory. It is appropriate to review at this time some of the policies initiated by Hale and the results that have followed from them. Hale's basic policy was to select fundamental problems and then build instruments to solve them, rather than to construct telescopes and then seek for problems to which they might be applied. The equipment of the new observatory was therefore planned with the solution of a few definite solar problems in view. Furthermore, Hale believed in making the attack on these problems of the sun from as many sides as possible. Since the sun is a star, this policy called for parallel studies of other stars. Thus during the first year of operation Hale used the Snow solar telescope and its spectrograph to obtain for the first time



high-dispersion spectra of a star for comparison with solar spectra obtained with the same instrument. With similar stellar observations in view, Hale almost immediately started construction of a 60-inch reflecting telescope using a disk which had been provided several years earlier by his father. For the same reason Hale initiated parallel studies of various phenomena in the laboratory for comparison with his solar observations. To this end a small physical laboratory was built on Mount Wilson and later a larger one in Pasadena. Studies in these laboratories of the spectra of various sources under different conditions of temperature, pressure, and magnetic fields in many cases provided the clues to the correct interpretation of the solar observations.

More recently, and to quite an extent through Hale's influence, the California Institute of Technology has developed large research laboratories in the physical sciences. Since the initiation of the joint operation of the Mount Wilson and Palomar Observatories, these laboratories have provided the desired close co-operation between astronomical and laboratory observations.

Throughout Hale's connection with the Observatory he stressed the importance of efficient instruments constructed to take advantage of the latest technical knowledge in the fields of optics and mechanics. To achieve this end, the telescopes and their auxiliary equipment were designed and built in the Observatory's own shops. Furthermore, instrument designers, opticians, and mechanics were retained on the permanent staff of the Observatory to make possible a continual revision of equipment as new advances in techniques became available.

In line with these policies Hale personally developed the tower telescope and the spectrohelioscope for the improvement of solar observations. He also saw the great possibilities of the reflecting telescope for astrophysical investigations. The development, under his direction, of the mirror

mounting and precision drives for the 60-inch and 100-inch telescopes eliminated the most serious disadvantages of earlier reflectors and led to the present almost universal use of this type of instrument. Later the design of the Ross corrector lens added still further to the effectiveness of the reflector by greatly enlarging its field for direct photography. This same policy regarding instrumentation encouraged the construction of the stellar interferometer by Michelson for use with the 100-inch telescope. It also led to the development by Strong of the aluminizing process for telescope mirrors. Very substantial increases in the speed and definition of stellar spectrographs have been achieved at the Observatory, first through the design of the Rayton and Bracey lenses, and later through Dunham's application of the schmidt camera to stellar spectrographs. This was followed by the development of various modifications of the schmidt camera to increase still further its speed and efficiency for this particular application.

The effectiveness of this close co-operation between the astronomer, the physicist, and the instrument designer which was a fundamental part of Hale's plans has been well substantiated by the results achieved at the Observatory during the past half century, first under Hale's own supervision and later under Adams' direction. As examples one may mention Hale's own discovery of the magnetic fields of sunspots and his clarification of the motions and other phenomena in the solar atmosphere.

The great light-gathering power and critical definition of the 100-inch telescope made possible Hubble's studies of the distances and structures of the galaxies, which showed that these are great stellar systems comparable to our own Milky Way system. These investigations completely revolutionized the concepts of the dimensions and content of the universe. Similarly, the development of the very high speed spectrographs permitted Humason to extend the measurements of the velocities of gal-

axies out to the most distant known clusters of these objects. Furthermore, the combination of Hubble's distances and Humason's velocities for these objects led to the formulation of the Hubble law of velocity as a function of distance and provided a quantitative basis for the concept of the expanding universe. In the spectroscopic field one important result was Adams' discovery and use of absolute-magnitude criteria to extend greatly our

knowledge of stellar distances. Similarly, the stellar interferometer yielded first in Michelson's and later in Pease's hands the only direct measurements that have been made of stellar diameters. Other studies too numerous to mention have been outlined in the Year Books of the Carnegie Institution and reported in detail in over thirty volumes of the Communications, Contributions, and Reprints of the Mount Wilson Observatory.

## OBSERVING CONDITIONS

The precipitation on Mount Wilson was again below normal, with a total of 29.93 inches. Observations were seriously interrupted between December 27, 1953 and January 4, 1954 by a forest fire which burned around the north, east, and south sides of Mount Wilson. In several places the flames came within a few hundred yards of observatory installations. No great hazard existed for the major telescopic installations, since they not only are of fireproof construction, but were also especially designed to protect the instru-

ments from heating effects of the sun. In some of the smaller nonfireproof installations, however, the hazards were considered great enough to warrant removal of optical parts to safe locations. The necessary readjustment of these optical parts after the fire caused still further delays in a few programs.

Solar observations were made on 340 days between July 1, 1953 and June 30, 1954. During this period observations were made on 246 nights with the 60-inch telescope and on 305 nights with the 100-inch.

## SOLAR RESEARCH

### SOLAR PHOTOGRAPHY

Solar observations were made by Cragg, Hickox, Nicholson, Richardson, and Dale Vrabec. The numbers of photographs of various kinds taken between July 1, 1953 and June 30, 1954 were as follows:

|   |        |
|---|--------|
| Direct photographs . . . . .                    | 670    |
| H $\alpha$ spectroheliograms, 60-foot focus . . | 312    |
| H $\alpha$ spectroheliograms, 18-foot focus . . | 1,002  |
| K2 spectroheliograms, 18-foot focus . .         | 993    |
| K2 spectroheliograms, 7-foot focus . . .        | 40,000 |
| K prominences, 18-foot focus . . . . .          | 1,035  |

### SUNSPOT ACTIVITY

The magnetic classification and study of sunspots and related phenomena have been continued by Nicholson and Cragg. Co-operative programs have been carried out with the U. S. Naval Observatory, the Observatory of Kodaikanal, the Meudon Observatory, the University of Michigan,

the Central Radio Propagation Laboratory of the National Bureau of Standards, and the Commonwealth of Australia Scientific and Industrial Research Organization.

During the calendar year 1953, solar observations were made at Mount Wilson on 350 days, 109 of which were without spots. The total number of spot groups observed in 1953 was 93, compared with 219 in 1952 and 294 in 1951. The northern hemisphere was slightly the more active in 1953, having 51 groups to 42 for the southern.

Since October 1953, solar activity has been at the minimum level, in fact a little lower than at most minima. Although 45 groups of the old cycle and only 4 groups of the new cycle were observed between July 1, 1953 and June 30, 1954, it is probable that new cycle spots will outnumber those of the old cycle by the end of 1954.



The monthly means of the number of spot groups observed daily for the past two and one-half years are shown in table 1.

No solar flares of intensity 1, two of intensity 2, and none of intensity 3 were recorded at Mount Wilson in 1264 hours of observing. The average number of flares per 100 hours of observing was 0.2 in 1953, 1.6 in 1952, and 2.3 in 1951.

TABLE 1

| MONTH                | DAILY NUMBER |      |      |
|----------------------|--------------|------|------|
|                      | 1952         | 1953 | 1954 |
| January .....        | 2.8          | 2.4  | 0.0  |
| February .....       | 2.0          | 0.5  | 0.1  |
| March .....          | 2.0          | 1.2  | 0.5  |
| April .....          | 2.2          | 2.3  | 0.3  |
| May .....            | 2.2          | 1.2  | 0.1  |
| June .....           | 3.8          | 1.9  | 0.0  |
| July .....           | 3.5          | 0.6  | ...  |
| August .....         | 5.1          | 2.0  | ...  |
| September .....      | 2.7          | 2.8  | ...  |
| October .....        | 2.0          | 0.8  | ...  |
| November .....       | 1.6          | 0.2  | ...  |
| December .....       | 3.1          | 0.3  | ...  |
| Yearly average ..... | 2.8          | 1.4  | ...  |

SUNSPOT POLARITIES

Magnetic polarities in each spot group have, if possible, been observed at least once. The classification of groups observed between July 1, 1953 and June 30, 1954 is indicated in table 2. "Regular" groups of

TABLE 2

| HEMISPHERE  | REGULAR   |           | IRREGULAR |           | UNCLAS-SIFIED |           |
|-------------|-----------|-----------|-----------|-----------|---------------|-----------|
|             | Old cycle | New cycle | Old cycle | New cycle | Old cycle     | New cycle |
| North ..... | 20        | 1         | 1         | 0         | 3             | 2         |
| South ..... | 17        | 1         | 0         | 0         | 4             | 0         |
| Whole sun.  | 37        | 2         | 1         | 0         | 7             | 2         |

the old cycle in the northern hemisphere are those in which the preceding members have S (south-seeking) polarity and the following members N polarity; in the

southern hemisphere the polarities are reversed. "Regular" groups of the new cycle are magnetically opposite those of the old cycle.

SOLAR ROTATION

A series of spectrograms are being obtained to determine the polar retardation of the solar rotation at minimum phase of solar activity, for comparison with later observations at sunspot maximum.

SOLAR MAGNETIC FIELDS

Harold D. Babcock and Horace W. Babcock have continued regular magnetic observations of weak fields of the sun at the Hale Solar Laboratory, using the solar magnetograph. Results to date, based on a sequence of 360 magnetograms, obtained in the pre-minimum years of the solar cycle, may be summarized as follows.

All the surface fields are found to have a fine structure, with local fluctuations in time that suggest a close connection with turbulence in the photosphere.

The main dipolar or "general" magnetic field of the sun, of average intensity one to two gauss and with polarity opposite to that of the earth, has been under observation for two years; it is usually limited to the polar regions in heliographic latitudes above  $\pm 60^\circ$ , but shows irregular fluctuations of intensity and extent. The main field is estimated to have a total magnetic flux of about  $3 \times 10^{21}$  maxwells and a moment of about  $1 \times 10^{32}$  gauss cm<sup>3</sup>. The external field, even at large distances, is probably far from that of a regular dipole, owing to perturbations by local low-latitude effects. No evidence has been found for obliquity of the magnetic axis to the axis of rotation, but an annual variation of the mean apparent flux ratio of the two poles, attributable to the varying heliocentric latitude of the earth, has been observed.

The numerous low-latitude magnetic fields of the sun are more or less localized and are transient, with areas ranging up to a fraction of a hemisphere, intensities up to several gauss, and lifetimes up to several



months. Most of the prominent magnetic areas may be classed as bipolar magnetic (BM) regions, although some are complex. Sunspots, when they occur, are within such regions. If the field intensity of BM regions is greater than about one gauss, they show a close identification with bright calcium flocculi or *plages* on spectroheliograms. The archlike patterns of bright coronal radiations reported by coronagraph observers are located above the stronger BM regions. This, together with evidence from eclipse photographs, suggests that the vertical extent of the BM fields is at least as great as their surface extent. There is a strong presumption that such coronal magnetic fields are important as sources of solar radio emission. The magnetic polarities of the preceding (west-erly) and following parts of BM regions in the northern and southern hemispheres, in a given cycle, obey the laws of polarity found by Hale for sunspots. The records usually suggest at least approximate equality of positive and negative magnetic flux in the respective parts. There is a great range in the total flux of different BM regions; a "typical" region had a flux of about  $1 \times 10^{21}$  maxwells.

A few interesting areas have been classed as "unipolar" magnetic (UM) regions, because the deflections are predominantly of one sign and it is not at all obvious where the emergent flux returns to the sun. The most prominent low-latitude UM region had, at central meridian passage on August 20, 1953, an area of more than 0.05 hemisphere, an intensity of about one gauss, and a flux of about  $1 \times 10^{21}$  maxwells. There is a good correlation of this UM region, on the six consecutive rotations of the sun on which it was observed, with the best 1953 sequence of terrestrial magnetic

storms of the 27-day recurrent type, and also with the best 27-day sequence of fluctuations of cosmic-ray intensity according to Dr. J. A. Simpson of the University of Chicago (unpublished). It is therefore suggested that the newly observed UM regions, as a class, may be identified with the heretofore hypothetical "M" regions of Bartels, which are believed to be the sources of solar corpuscular streams.

A comparison of hydrogen spectroheliograms with the magnetograms suggests that the stable dark filaments (prominences) tend to be situated near the borders of BM regions, partly encircling them, or, alternatively, to lie along a line dividing the BM region into two parts of opposite polarity.

The magnetic pattern of the sun shows very considerable changes from day to day, and there is evidence for appreciable fluctuations, of the order of one gauss, in a few minutes. Such fluctuations are plausibly related to a rather large-scale magnetodynamic turbulence of the photospheric material; hence fluctuating velocity fields on a similar scale may be expected. The apparatus, readily converted for sensitive measurement of the Doppler effect, records velocity traces across the disk in 5 minutes with a precision of 10 meters per second. Preliminary results indicate that differential velocities of order 100 m/sec are characteristic of areas 15,000 km in extent.

Because of current interest among theoreticians in the behavior of convective turbulence in the presence of a magnetic field and under the influence of the Coriolis force, special emphasis is planned on further observations of related magnetic and velocity fields, and on the development and life history of BM and UM regions.

## PLANETS AND SATELLITES

Photographs of Mars, Jupiter, and Saturn have been taken by Pettit and Richardson with a motion-picture camera attached at the Cassegrain focus of the 60-inch tele-

scope and the coudé focus of the 100-inch. At the 60-inch a negative lens was used to make the scale equal to that at the 100-inch without enlargement, or  $1 \text{ mm} = 2''.7$ . At

opposition this scale gave an image of Mars 8 mm in diameter. The times of exposure for Mars made on a fine-grain emulsion ranged from 0.8 second in blue light at the 60-inch to 0.2 second at the 100-inch in yellow light.

On runs made during twenty nights from April 17 through July 4, 1954, 380 exposures were obtained of Jupiter, 3400 of Saturn, and 8100 of Mars. The images of Jupiter in blue and yellow light show much interesting detail. The best images of Saturn taken in integrated light show Encke's division and division no. 4 in the rings, markings often seen but rarely photographed.

#### OBSERVATIONS OF MARS AT THE 1954 OPPOSITION

The blue images of Mars taken on June 2 and 3, when viewed with the south pole uppermost, show a marking near the equator roughly resembling the letter W. The marking is presumably a cloud formation in the upper atmosphere of the planet. It is on the receding half of the disk between areographic longitudes  $66^\circ$  and  $120^\circ$ . The marking does not show on the yellow images taken on these nights, or on maps of the planet. Round knobs at the ends of the stroke of the W nearest the central meridian are about 300 miles in diameter and are joined by a broad line 1100 miles long. The more conspicuous knob at  $118^\circ$ ,  $12^\circ$  S, is 35 per cent brighter than the disk around it, and 70 per cent as bright as the south polar cap. Measures on three of the best-defined points of the W show it to be moving at essentially the same rate as that at which the planet is rotating.

Markings on the yellow images which are easily visible on dozens of exposures occur at about the same positions as the canals Antaeus, Cerberus, Hades, Eunostos, and Hyblaeus. On the photographs,

however, these markings appear as irregular streaks or broad bands rather than as the lines shown on most drawings and maps of the planet.

A large irregular area resembling the dark blue-green maria has appeared in the northern hemisphere between  $230^\circ$  and  $270^\circ$ , which seems to be a development of the fine complicated structure northeast of the Syrtis Major. Photographs and drawings obtained at other observatories at former oppositions show this region as practically blank.

Photographs of Mars in blue light are usually featureless except for bright caps over the poles. Occasionally, however, the atmosphere becomes exceptionally transparent in blue light, so that the surface markings can be discerned. There is evidence that these periods of blue clearing, which may last for several days, are most likely to occur around the time of opposition. Photographs in blue light on June 25 and 26 (opposition date June 24), although taken in poor seeing, unmistakably reveal the Syrtis Major and adjacent surface markings; the closest observation previous to this date was June 3. The next observations, on June 30, taken during fairly good seeing, show the Syrtis Major as well as the Trivium Charontis. On the remaining photographs through July 4 these markings become gradually fainter in blue light. Thus these observations, although incomplete, indicate that blue clearing occurred during a few days around opposition, and that it is a phenomenon of the Martian atmosphere which takes place gradually rather than abruptly.

#### JUPITER'S SATELLITES

Photographs were taken by Nicholson to determine the positions of Jupiter's faint satellites at the 1953-1954 opposition of Jupiter.

#### STELLAR SPECTROSCOPY AND PHOTOMETRY

About one-half of the observing time of the 100-inch and 200-inch telescopes has

been devoted to spectroscopic investigations. Because of the search for novae in



the galaxy in Andromeda with the 60-inch, the use of this instrument for spectroscopic observations has been limited to a much smaller fraction of the total observing time. During the report year 90 spectrograms have been obtained with the 60-inch, 920 with the 100-inch, and 640 with the 200-inch Hale telescope. Most of the observations with the 200-inch have been concentrated on faint objects which the great light-gathering power of this instrument and the high efficiency of its spectrographs make it possible to observe effectively for the first time. These include white dwarfs, subdwarfs, and stars in globular clusters which are the brightest definite representatives of population II.

#### VARIABLE STARS

The spectrograms of  $\alpha$  Ceti (Mira) obtained from 1934 to 1952 by several observers with the coudé spectrographs of the 100-inch and Hale telescopes have been examined by A. H. Joy. With dispersions up to 2.3 Å/mm the spectrographic behavior of the variable star was checked in greater detail than was previously possible.

The measures of emission lines on 88 plates show a maximum positive velocity near minimum light, whereas the absorption-line velocities are highest near maximum light. The longward displacement of the bright and dark lines of iron is greater for atoms of higher excitation potential. Emission lines of numerous elements were identified, but several strong lines from unknown sources were observed. In certain cycles sharp forbidden lines of iron were found at phases preceding minimum light. The velocities yielded by the absorption lines vary slightly for different elements.

Spectrographic observations of the peculiar variable star AE Aquarii made by Joy during the past ten years afforded definite evidence that it is a most unusual spectroscopic binary. One component of the pair is evidently a main-sequence star having about the mass and diameter of the sun and an absorption-line spectrum of

type dKo; the other is a hot subdwarf only one-twelfth as large but having nearly the same mass. Spectra of the hot star show variable emission lines of hydrogen, helium, and ionized calcium, indicating a surrounding envelope which is expanding at a rate of 750 km/sec. The intensity of the strong ultraviolet continuum varies with that of the bright lines. The star is of fainter visual luminosity than the Ko star.

The period is 0.70 day, and the velocity range is about 300 km/sec for each component. The peculiar flat maxima of the velocity-curve of the Ko star may be accounted for by heating or reflection effects of radiation from the hot star falling upon one hemisphere of the cooler star, whose rotation period is nearly equal to the orbital period.

A three-color photoelectric light-curve has been nearly completed by Arp for the prototype type II cepheid W Virginis. This will supplement the extensive spectroscopic analysis recently completed by Helmut Abt. Preliminary reduction confirms the large time delay of light-curve features in successively longer effective wave lengths, as observed in the globular-cluster cepheids. The observed minimum occurred approximately one day later in each of the three colors, U, B, and V.

Simultaneous photoelectric and spectroscopic observations of Eggen's rapid variable star BD + 41° 119 have been obtained by Wilson and Walker. This star is probably a representative of the  $\delta$  Scuti class of variables, characterized by periods of from 2 to 4 hours and spectral classes in the early F's. The photometric observations were made using the 20-inch reflector on Palomar, and the spectroscopic observations were obtained with the coudé spectrograph of the 200-inch reflector. The faintness of the star ( $m_v=8.5$ ) and the shortness of the period (approximately 3 hours) require the use of the large light-gathering power of the 200-inch telescope in order that the exposure times of the spectroscopic observations may be made short enough to resolve the rapid variations in velocity.



Combining the results, it is found that the light- and velocity-curves are mirror images of each other, as in the cases of  $\delta$  Scuti and the cepheid variables. The observations will be used to apply the semiempirical tests of pulsation to this star.

Velocity-curves have been obtained of HD 223065, another of Eggen's short-period variables, by Wilson.

The discovery of extremely rapid light-variations in MacRae  $+43^\circ 1$  has brought to light an entirely new type of light-variation. The variability of this star was originally discovered by P. P. Parenago in 1946. He classified it as an irregular variable having a range of 0.9 mag. Walker's observations of this star with the 100-inch reflector have revealed the existence of extremely rapid variations in light, amounting, in the ultraviolet, to as much as 0.4 mag. in 5 minutes. The star is continuously variable, the periods of the fluctuations ranging from 1 to 30 minutes.

Fluctuations are present in each of the three colors, the amplitudes in the yellow and blue being 0.7 and 0.8, respectively, of the amplitude in the ultraviolet. On August 9, 1953, the colors of the star on Johnson and Morgan's system ranged from  $B-V = -0.13$ ,  $U-B = -1.05$  at one of the light-maxima to  $B-V = -0.08$ ,  $U-B = -0.98$  at one of the light-minima. The star is abnormally bright in the ultraviolet for its  $B-V$  color, and in Johnson and Morgan's diagram for  $B-V$  versus  $U-B$ , it falls off from the relationship for normal main-sequence stars and lies just at the blue end of the region populated by the white dwarfs. The fact that Greenstein finds no interstellar K line in the spectrum seems to indicate that the effect is intrinsic rather than being caused by interstellar reddening; the star is thus similar to but somewhat hotter than the white dwarfs. Greenstein concludes that the luminosity of the star is  $M_v \geq +3$ , and finds that the spectrum of the star resembles that of an old nova. These facts, together with the instability of the star (shown by the light-variations) and the similarity to the white

dwarfs, suggest that  $+43^\circ 1$  is in some way related to the novae; it may be an old nova or perhaps a "pre-nova" which has not yet flared up.

If  $+43^\circ 1$  is an old nova or a pre-nova, then we might expect to observe similar short-period light-variations in the known old novae. Twelve of the brightest of these stars have now been examined by Walker for short-period light-variations: N Persei 1901, T Aurigae, N Geminorum 1912, T Coronae Borealis, N Ophiuchi 1848, RS Ophiuchi, DQ Herculis, N Aquilae 1918, N Lyrae 1919, N Sagittae 1913, Q Cygni, and N Lacertae 1910. Eight of these have been definitely shown to exhibit the same type of short-period light-variation displayed by  $+43^\circ 1$ . The remaining four, T Aurigae, N Geminorum 1912, N Ophiuchi 1848, and N Lacertae 1910, either were constant or had light-variations of only a few hundredths of a magnitude at the time of observation.

The application of this criterion to stars suspected from their spectra or for other reasons of being old novae is obvious. Three such suspected stars have been observed for light-variations: (1) V426 Ophiuchi, which was found by Herbig to have a spectrum resembling that of an old nova; (2) EM Cygni, whose spectrum was found by E. M. and G. R. Burbidge to be similar to that of an old nova; (3) V Sagittae. The stars V426 Ophiuchi and EM Cygni both displayed the short-period light-variations; V Sagittae was either constant or variable by only a few hundredths of a magnitude at the time of observation. Thus, the first two very probably are old novae, but the absence of light-fluctuations in V Sagittae does not necessarily mean that it is not an old nova.

Observations have also been made of SS Cygni which show that it too undergoes these extremely rapid fluctuations in light. This result may provide new evidence for a relationship of some sort between the SS Cygni stars and novae.

Simultaneously with Walker's photoelectric observations of T Coronae Borealis,

Deutsch obtained near-ultraviolet spectra at a dispersion of 110 Å/mm. The observations would have revealed any pronounced changes in the line spectrum that might accompany the brightness variations which Walker has found to occur from minute to minute. The results are negative, and suffice to show that the brightness changes cannot be attributed to fluctuations in the emission lines or in the continuous Balmer emission.

The visual light-curve of Nova Puppis 1942 has been brought up to date by Pettit. The rate of decline is about 0.35 mag. per year, and the present brightness is 13.3 mag. In all, a total of 25 comparison stars have been measured for the photometry of this field.

A supernova in NGC 5668 found by Wild with the 18-inch schmidt telescope was observed by Deutsch near maximum brightness with a dispersion of 38 Å/mm at the coude spectrograph of the 200-inch telescope. Other than the wide bands that Minkowski has described in earlier supernovae of type I, this spectrum showed no certain discernible features except two sets of interstellar  $Ca\ II$  lines. One set, nearly undisplaced, is produced by the gas in our Galaxy; the other set, of comparable strength, is displaced longward by 1600 km/sec and must be attributed to the interstellar gas in NGC 5668. The Hubble type of NGC 5668 is Sb.

The spectra of a supernova in NGC 4214, also discovered by Wild, were observed by Minkowski; though the spectra showed the general characteristics of the supernovae of type I, they were distinctly different in several details from those of earlier examples. At this time, it is not possible to decide whether this supernova is actually different from other known supernovae or whether the spectra merely show a phase, probably about six weeks after maximum light, which has not been adequately observed in previous supernovae of type I. The brightness of the supernova at the time of discovery admits such an interpretation.

#### GLOBULAR AND GALACTIC CLUSTERS

With the aid of the coude spectrograph of the 200-inch Hale reflector, Greenstein has studied at 18 Å/mm, using exposures of one or two nights, the bright K giants in the globular clusters M 13 and M 92. Stars in both clusters show clear evidence that their excitation temperature is lower than previous estimates based on spectral classification alone would indicate, and in better agreement with the colors. In particular, lines from the ground states of the metals are relatively very strong, in some cases reaching intensities like those in an early M star. It is possible that the stars have very extended atmospheres with large temperature gradients, and that effects like those in "shells" begin to appear. The stars in M 92 had previously been called F stars, but they are clearly of later type, with excessively weak lines. Although very elaborate analysis will be needed to obtain the abundance ratio of the metals to hydrogen, it appears that a low abundance of metals may still be found, since the effect of a "shell" is mainly to increase resonance-line intensities. A serious complication occurs only if the shell is optically thick. It could then suppress lines from the normal reversing layers, and probably also distort the continuous energy distribution.

The blue stars in the horizontal branch of the globular clusters M 3 and M 13 have been observed by Münch. The spectra so far obtained indicate that even at the small dispersion of 240 Å/mm it is possible to recognize characteristics of these stars that distinguish them from type I blue stars.

Spectrograms suitable for classification have been obtained by Deutsch of about 24 red giants in the bright globular clusters M 3, 5, 13, 15, 22, and 92, using the 100-inch and 200-inch telescopes. These spectrograms confirm that within each cluster the spectra are homogeneous with respect to the strength of the metallic lines and of  $CH$ , but that these parameters vary widely from cluster to cluster. Only the M 15



stars show the pronounced weakness of the metallic lines discovered by Baum in M 92. It may be significant that among the seven clusters photometered by Arp, Sandage, and Baum, the bluest red-giant branches occur in just these two. If the weakness of the metallic lines in M 15 and M 92 is a true abundance effect, it indicates a metal deficiency of the order of 10 times, as contrasted with the deficiency of the order of 2 times found in some high-velocity stars of the solar neighborhood.

Walker has continued his program for the detection of incipient variability of stars at the edge of the variable-star gap in M 3 and M 5. During the summer of 1953, 18 stars lying at either edge of the variable-star gap in M 92 were observed for variability. The probable error of a single observation was about  $\pm 0.02$  mag. Also observed were 3 stars suspected of variability by J. J. Nassau, N-14, N-15, and N-16. N-14 was the only star of the 21 observed which showed any detectable variation, i.e., greater than 0.01 or 0.02 mag. The absence of variability in the other stars confirms the preliminary finding obtained last year for M 3 and M 5, that the onset of variability is very abrupt at the edges of the variable-star gap.

Observations of selected red giant stars in M 3 and M 92 for variability, begun in 1953, have been continued and completed during 1954; reduction of the observations is not yet complete.

Sandage and Morton S. Roberts have completed their study of the variables in M 3. Light-curves in two colors ( $m_{pg}$  and  $m_{pv}$ ) for 52 RR Lyrae variables were obtained. These data permitted the variables to be plotted in the color-magnitude diagram previously obtained for the nonvariable stars in this cluster. All the variables were found to lie in a sharply delineated region unoccupied by nonvariable stars. This result confirms similar data obtained by Martin Schwarzschild in 1940.

Three stars previously believed to be nonvariables fell inside the region occupied by the RR Lyrae stars. On inspection,

these three stars proved to be variables of small amplitude. One of them fell on the blue edge of the gap, with a period of 0.28719 day and an amplitude of 0.16 mag. The other two fell near the red boundary, with periods of 0.9170 and 0.9987 day and amplitudes of 0.14 and 0.15 mag.

These three stars are consistent with the general color index-amplitude relation found to hold for all cluster-type variables studied. This relation shows that the amplitude of variation is small at each edge of the variable-star region and rises to a maximum of 1.6 mag. in  $m_{pg}$  at C.I.=0.07.

The mean magnitude and color index were used to test the pulsation criterion  $P\rho^{\frac{1}{2}} = \text{constant}$ . The data are entirely consistent with theory in giving the correct relation between the observed quantities of  $\log P$  vs. C.I. The type  $a$  and  $b$  variables define the required linear relation, and the type  $c$  stars define a second relation with the same slope but displaced in period by  $P_a, b/P_c = 1.48$ . This same slope indicates that the type  $c$  variables also satisfy the pulsation criterion. Their displacement in period could indicate pulsation in a different mode from that of the  $a$  and  $b$  stars. The observed frequency ratio of 1.48 is near the theoretical ratio of the second mode to the fundamental in pulsation models computed by I. Epstein.

As a continuation of the earlier study of type II cepheids in globular clusters, three-color photoelectric light-curves are being derived by Arp for four of those cepheids, two in M 5 (25.7 and 26.5 days) and two in M 10 (7.9 and 18.8 days).

In addition, long photographic runs have been compiled on the cepheids in two new clusters, M 22 and M 56, as well as in three previously investigated, M 2, M 3, and M 5. This work will supplement the earlier data, check secular changes, and furnish more data on the alternation of cycles in some of the variables.

Three-color photoelectric sequences have been established in the globular clusters M 22 and M 56 by Arp. Plates with the 60- and 100-inch telescopes have been as-



sembled for these clusters preparatory to deriving color-magnitude diagrams in three colors and hence the cluster moduli and luminosities of the cepheids and long-period variables.

In co-operation with H. L. Johnson, who has supplied faint photoelectric comparison stars in three colors, and with Sandage, who has obtained 100-inch plates, Arp has extended the M 13 color-magnitude diagrams to about  $V=18.4$  in all three colors. The  $B-V$ ,  $U-B$  relation for the M 13 giant sequence agrees well with that for the unreddened yellow giants and the main sequence in the vicinity of the sun. Below about  $M_V=+2$ , color-magnitude measures have been extended very close to the center of M 13 and farther to the outside than in the original measures. Some evidence is found for differences in distribution and color of stars with radius; at this point, however, photometric effects cannot be completely excluded, and variable obscuration as well as intrinsic changes in the stars as a function of radius is possible.

Some photoelectric measures have been made by Arp with three colors on the integrated magnitudes of two globular clusters. In both M 2 and M 3 there appears to be a small but definite reddening toward the center of the cluster.

Osterbrock obtained blue and yellow plates with the 100-inch telescope to be used in the construction of the color-magnitude diagrams of the globular clusters M 15 and NGC 7492. Additional plates will be taken in the coming season, including plates with the 200-inch telescope of M 15, to push the observations in that cluster to as faint a limit as possible.

Three-color photometry on the intrinsically faint globular cluster NGC 4147 was completed by Sandage and Walker. This cluster had previously been reported in the literature as peculiar, since it appeared to have no giant stars brighter than  $M_V=-0.5$ . In normal globular clusters the giant stars range in absolute magnitude from  $M_V=0.0$  to  $M_V=-3.0$ . Differences

between globular clusters are of great importance, because they are the observational manifestation of perturbations in the evolutionary process which takes stars from the main sequence and moves them into the giant region of the Hertzsprung-Russell diagram. The present three-color observations on NGC 4147 failed, however, to confirm the peculiarities reported in the literature. The color-magnitude diagram on the  $B$ ,  $V$  photometric system of Johnson and Morgan shows nothing at all unusual about the giant sequence. The giant branch is present and is populated at the normal relative frequency. The luminosity function of NGC 4147 is nearly identical with that of M 3 except for a scale factor of  $1/10$ . The similarity of both the color-magnitude diagram and the luminosity function for the giant sequences of NGC 4147 and M 3, together with the general similarity at the bright magnitudes of additional globular clusters studied by Arp (M 2, 5, 10, 13, 15), implies that the evolutionary tracks for these types of stars show no large dissimilarity.

The two-color-index diagram  $B-V$  vs.  $U-B$  showed that for stars in NGC 4147 redder than  $B-V=0.5$  an ultraviolet excess of about 0.5 mag. exists. The ultraviolet excess disappears for stars on the horizontal branch with  $B-V$  ranging from 0.2 to  $-0.1$ . A similar situation is known in M 3 from co-operative work by H. L. Johnson at Lowell Observatory. These data suggest a low metal abundance for globular-cluster stars as compared with stars in the solar neighborhood. For cool stars ( $B-V>0.5$ ) the blanketing effect of the metal lines on the continuous radiation below wave length 3800 Å will be less if the metal abundance is low. For hotter stars ( $B-V<0.2$ ), where few metal lines are present anyway, the relative lack of metals will have little effect, and no great excess in the ultraviolet would be expected. If further work shows that the ultraviolet excess is a common property of all globular-cluster stars, we have a photometric method of selecting field stars with some of the same

characteristics as stars in globular clusters. An incidental result of the work on NGC 4147 was the discovery of 7 new RR Lyrae variables, making a total of 11 now known in this cluster.

It was reported in last year's annual report (Year Book No. 52) that tentative measures by Baum of two faint stars in M 13 seemed to place them unexpectedly far below the familiar main sequence in the color-magnitude diagram, and that this discovery presented a puzzle as to their identity. It now turns out that these stars and others measured subsequently by Baum constitute the main sequence of M 13 and that they lie fully 2 magnitudes below the ordinary main sequence defined by stars in the solar neighborhood, if the cluster-type variables in M 13 are adjusted to an absolute magnitude around  $M=0$ . Alternatively, if the main sequence of M 13 were assumed to coincide with that for the solar neighborhood, the cluster-type variables would have to be placed at  $M=-2$ , thereby upsetting the cosmic distance scale again. Several arguments, based in part on comparison of the color-magnitude diagram for M 13 with those for other globular clusters, favor keeping the cluster-type variables near  $M=0$  and consigning the main sequence of M 13 to the so-called subdwarf region of the color-magnitude diagram. M 13 is the first population II system whose main sequence has been determined with sufficient reliability to justify firm conclusions.

This result is of fundamental importance for two reasons. First, it demonstrates that the position of the main sequence is far from being unique. In other words, the traditional main sequence defined by the near-by stars with which we are intimately familiar represents only the particular stellar population (population I) in our own region of the Galaxy and does not apply universally in even an approximate way. In the second place, it identifies the so-called subdwarfs as main-sequence stars of population II. Owing to the probable large-scale preponderance of population II,

the subdwarfs, instead of being unusual objects, may in reality be the most numerous stars in the universe.

Baum has made similar photoelectric determinations of the magnitudes and color indices of a few stars in the main sequence of M 3. These preliminary results indicate that the departure from the population I main sequence by the stars in M 3, though probably real, is much smaller than in M 13. Additional extensive measurements in M 3 are planned for the coming observing season.

The evolutionary tracks in the Hertzsprung-Russell diagram which stars in the globular cluster M 3 have followed have been computed semiempirically by Sandage. The tracks were constructed from the observed luminosity function and the observed color-magnitude diagram for M 3, both of which had been obtained last year. It is assumed that all stars now on the subgiant, giant, and horizontal sequences came originally from the main sequence and have evolved from their initial state as a result of interior structure changes caused by the conversion of hydrogen into helium. If the initial process of their evolution follows the theoretical tracks of Schönberg and Chandrasekhar (until 12 per cent of the hydrogen is burned), the data implicit in the luminosity function and the sequences in the color-magnitude diagram permit the construction of the complete life history of the star. The rate of evolution along the tracks and the amount of hydrogen burned at each stage have also been obtained. The results of the work indicate that by the time a star has reached the top part of the giant branch at  $M_v \approx -3.0$ ,  $C.I. \approx 1.5$ , it has consumed most of its hydrogen. It seems likely therefore that stars on the horizontal branch have burned nearly all their fuel and consist mainly of helium interiors with only an outer hydrogen envelope. As the interior structure of stars on the horizontal branch approaches chemical homogeneity, the stars approach the main sequence again. Finally, as all the fuel is burned, the star sinks in luminosity pre-



sumably into the pre-nova state and subsequently becomes a white dwarf.

Partial confirmation of the validity of the evolutionary tracks comes from data on the masses of stars. The tracks of evolution are also the locus lines of constant mass. Thus, by this theory, the mass-luminosity relation for the globular-cluster stars in M 3 has been predicted. The field star  $\zeta$  Herculis A, the mass of which is accurately known, lies on the color-magnitude diagram in the subgiant sequence of M 3. The M 3 evolutionary track passing through this point refers to a star of mass  $1.17 M_{\odot}$ . The mass of  $\zeta$  Herculis A observed by R. G. Hall is  $1.12 M_{\odot}$ . The usual type I mass-luminosity relation predicts  $1.60 M_{\odot}$  for  $\zeta$  Herculis A. The predicted mass of evolutionary theory agrees well with the observed mass. This agreement is taken as partial confirmation of the validity of the derived paths taken by these type II stars as they age.

The co-operative program between H. L. Johnson and Sandage on the rich galactic cluster M 67 has been completed. Magnitudes in three colors for about 500 stars were determined. The apparent distance modulus determined by fitting the color-magnitude diagram to the data for near-by stars is  $m-M=9.6$ . This cluster has no main sequence brighter than  $M_V=+3.0$ . The cluster has a heavily populated giant and subgiant sequence, however, joining the main sequence at  $M_V=+3.0$ . The cluster diagram is what would be expected in an old aggregate of stars where stellar evolution has taken place according to the general thesis developed by Sandage and Schwarzschild. The time since all stars in M 67 were on the main sequence is about  $3 \times 10^9$  years by the dating procedure within the framework of the evolutionary theory. This is one of the oldest galactic clusters known. It is also one of the densest known. It is so dense that it could have survived for  $3 \times 10^9$  years against the dispersive forces acting on clusters in the Galaxy.

There are differences between the color-

magnitude diagrams of M 67 and M 3, undoubtedly reflecting the effect of chemical composition on the evolutionary tracks in the Hertzsprung-Russell plane. Popper's spectra of the giant stars in M 67 show them to be normal-luminosity class III giants with no large metal deficiencies. On the other hand, the M 3 spectra indicate abnormal metal abundance. Furthermore, M 67 has a normal B-V vs. U-B diagram, indicating no ultraviolet excess, in contradistinction to M 3. These data suggest that metal abundance has a large effect on the detailed track of evolution for individual stars after they leave the main sequence.

Sandage is undertaking a theoretical study of the evolutionary tracks in the Hertzsprung-Russell diagram of M 67, similar to the study of M 3 reported above. A comparison of the tracks of M 67 stars with those of M 3 stars should provide direct observational evidence for the dependence of the details of evolution on chemical composition.

Deutsch and Walker have investigated the galactic cluster NGC 457 in order to check the results of W. Becker, who found, in this and several other clusters containing B stars, that the brightest stars have Balmer discontinuities which are abnormally large for their spectral class. Using the 20-inch and 100-inch reflectors, Walker made three-color photoelectric observations of approximately 75 cluster stars. These observations do not appear to confirm the existence of abnormally strong Balmer decrement in the stars of this cluster. Spectra for some 20 stars of types earlier than A0 have been obtained by Deutsch. Except for one B5 supergiant, the most luminous stars in this cluster are early B giants, of luminosity class III, which show a marked tendency for the earlier Balmer lines to go into emission.

The color-magnitude diagram of the nebulous cluster NGC 2264 has been investigated by Walker to  $V=17$  in order to ascertain the stellar contents of a cluster containing both O and B stars and a large



number of faint variable stars, many of which have been shown by Herbig to be emission-line stars. These are presumably T Tauri type variables. The existence of an O7 star (S Monocerotis) in this cluster, as well as a number of B stars, indicates that the age of the cluster is only a few million years. NGC 2264 is one of the most favorable objects of this type for investigation, since, even though the stars are associated with an extensive dark cloud, the total interstellar absorption is only 0.1 mag. and appears to be fairly uniform.

Photoelectric observations of the cluster on the three-color system of Johnson and Morgan have been obtained with the 60- and 100-inch reflectors of the Mount Wilson Observatory, and yellow, blue, and ultraviolet plates have been obtained with the 60-inch reflector for supplemental photographic photometry.

A preliminary color-magnitude diagram indicates that there is a continuous sequence of stars connecting the O and B stars and the T Tauri stars, the latter appearing abruptly at  $V=13$ . Below A0, however, the sequence departs from a standard main sequence by first rising  $1\frac{1}{2}$  mag. and then extending as a broad band, 2 mag. wide, parallel to and about 2 mag. above the normal main sequence. The reality of the  $1\frac{1}{2}$ -mag. rise has been confirmed by spectroscopic observations; the stars at the beginning of the rise average about A0V, whereas those at the top of the rise are about A5III. Below  $V=13$  the *maximum* departure of the stars from the main sequence increases, reaching about 6 mag. at  $B-V=1.6$ . Whether the mean line also diverges is not clear owing to the incompleteness of the observations. The luminosity function, which is complete to  $V=17$ , shows a peak at  $V=15$ . Assuming that the upper portion of the main sequence is normal, the distance modulus found by matching Johnson and Morgan's main sequence to the cluster sequence is 10.4 mag., or 1200 parsecs.

Theoretical studies by various workers have suggested that in a young group of

stars such as NGC 2264 the stars of smaller mass may still be in the process of contracting gravitationally from the pre-stellar medium, and as a result should populate a sequence of decreased slope diverging from the main sequence at a point which depends on the age of the group. Though the divergence of the lower portion of the cluster sequence is not precisely of the form predicted by theory, the general departure of the stars from the main sequence is in the direction required for stars which are still contracting from the pre-stellar medium. Consequently, it is possible that we are observing in this cluster the actual formation of stars out of the dark interstellar material with which the cluster is associated. If the point at which the cluster sequence departs from the main sequence is at A0, then according to the calculations of Salpeter the age of the cluster is about  $10^6$  years, which is consistent with the age inferred from the presence in the cluster of the O7 star. The proper-motion data now available are not adequate for an attempt to determine the age of the cluster by possible motions of expansion.

About 30 new variable stars have been found in the cluster during this investigation, bringing the total number of known variables to about 60.

#### WHITE DWARFS AND SUBDWARFS

Spectrophotometry of white dwarfs, at 38 Å/mm, has been completed by Greenstein for 12 stars. The most interesting new feature detected is the great broadening of the *Fe I* lines in the ultraviolet spectrum of the cool white dwarf Van Maanen 2. This object probably has a mean density of 10 tons per cubic inch. The velocity of escape is near 5000 km/sec. Under these conditions, instead of the many sharp lines of *Fe I* seen in normal stars, the pressure broadening produces broad blends up to 50 Å wide, with the deduced half-width of single lines near 8 Å. This, if interpreted as collisional broadening, corresponds to a damping constant 60,000 times

the classic radiation-damping width. The interpretation must be refined by use of a model atmosphere, since the effect of integration through the outer layers is undoubtedly such as to give too high an apparent collisional-broadening coefficient.

An unexpected phenomenon in four white dwarf spectra has been the presence of unstable envelopes at relatively low pressure. Three objects are of the helium-rich type, and show variable sharp lines at large negative velocities. In Van Maanen 2, there are strong, sharp cores asymmetrically placed in the broad H and K lines of Ca II. The cores have a mean velocity of +36 km/sec; underexposed plates, which presumably give more nearly the center of the broad H and K, yield a velocity of +114 km/sec. Old plates at very low dispersion gave +238 km/sec (R. E. Wilson). At the moment nothing can be deduced about the Einstein shift for these objects, nor about the true velocities of the stars. A serious theoretical question arises as to the nature of the forces which can expel gas from stars of such high surface gravity. The compressed residual magnetic fields seem most promising.

The survey of subdwarfs is being continued by Greenstein. About 75 suspected or probable subdwarfs, from O8 to G8, have been observed at 18 A/mm. Many prove to be weak-lined high-velocity stars, but others show extreme line weakening, and sharp lines of H (or of He at high temperature), and are probably far below the main sequence. Preliminary results indicate that hot stars, particularly, exist with a wide variety of peculiarities. The Humason-Zwicky faint blue stars of type B3 to A0 are relatively normal, although possibly underluminous. They have only moderately large radial-velocity dispersion (38 km/sec). The earlier-type stars are more peculiar, and include a hot absorption-line, carbon-rich star (HZ 15), several helium-rich subdwarfs (HZ 1 and HZ 44), an old nova (MacRae +43°1), and a helium-rich possible white dwarf (HZ 3).

### STELLAR MAGNETIC FIELDS

Spectroscopic observations of selected magnetic stars have been continued as regularly as possible by H. W. Babcock. The gradually accumulating data on magnetic variations and related phenomena make possible the discussion of preliminary elements of variation of these stars, one by one, or show for some that the variations are irregular. Those receiving most attention recently have been HD 71866, HD 188041,  $\alpha^2$  Canum Venaticorum, and  $\beta$  Coronae Borealis. The large and very rapid changes in the magnetic field of HD 71866, as well as its extraordinary crossover effect, indicate that it deserves much further study. Line-intensity variations of the rare-earth elements and of Sr II have been discovered in HD 188041; they precede in phase the nonuniform magnetic variations in the 226-day period. The readily measurable magnetic field of  $\beta$  Coronae Borealis varies quasi-periodically in about 50 days, usually between the limits -1000 and +2500 gauss, but with occasional large discrepancies in amplitude. Growing evidence suggests more strongly that in 78 Virginis and HR 710 the pronounced magnetic fluctuations are irregular. Magnetic fields have been discovered in Brewer's star (HD 50169) and in Abt's star (HD 98088).

Some arguments have been given by Deutsch for interpreting H. W. Babcock's observations of periodic magnetic variable stars in terms of the "oblique rotator" model, in which the magnetic and rotational axes of the star do not coincide. By idealizing a real magnetic star as a perfectly conducting, sharply bounded, rigidly rotating sphere in a vacuum, it has been possible to establish that the electromagnetic field equations are not incompatible with an oblique-rotator type of model. The field external to such an idealized magnetic star has been worked out in detail for the case of a dipole magnetic field on the surface. In the idealized model, the external electric field intensity has the in-



interesting property that near the star it has an appreciable component along the lines of force of the magnetic field, and therefore is capable of accelerating ions to very high energies. The conductivity of the matter surrounding a real star will, however, completely alter the character of this external field, and inhibit the acceleration of circumstellar ions, unless the density is much lower than one would expect it to be.

#### PHOTOMETRY OF SELECTED AREAS

The program for obtaining accurate photoelectric magnitudes in a series of Selected Areas has been practically completed by Baum. These photoelectric standard sequences span a range of ten or more magnitudes with an average of four stars per magnitude in each of ten Selected Areas. In general, conventional d.-c. techniques were used for covering the range from roughly 9th to 19th magnitude, but in three of the most favorably situated areas the sequences were extended during the past year to 22d or 23d magnitude by means of the new photoelectric photon counter described in the last annual report. Reduction of the observational material to magnitudes and color indices on the International System is also complete; it remains only to assemble the material into usable form and to make certain final adjustments. The merit of these photoelectric standard sequences, of course, is that they are free of the large systematic scale errors which formerly plagued photographic work; this not only is assured by the inherent linearity of the photoelectric process, but also has been checked by experiments both in the laboratory and at the telescope.

#### OTHER STUDIES

An analysis of the rotation of the near-by A and F giants has been completed by Greenstein and J. B. Oke. Many of these stars, located in the Hertzsprung gap, are found from Mount Wilson coude spectra to be rotating rapidly. Consideration of

possible evolutionary tracks suggests that rotating A and F giants must originally have been much more rapidly rotating B or A main-sequence stars. They cannot have evolved from the late-type stars, because of the absence of rotation in the later types.

A general review of nuclear reactions affecting the abundances of the elements has been made by Greenstein. The major effects expected at low temperatures appear in the changes of abundance ratios of  $H/He$  and  $H/metals$ , the destruction of  $D$ ,  $Li$ ,  $Be$ , and  $B$ , and the variation of  $He^3/He^4$ ,  $C^{12}/C^{13}$ , and  $C^{12}/N^{14}$ . At higher temperatures  $C^{12}$ ,  $O^{16}$ , and  $Ne^{20}$  may be formed, and neutrons may be produced from the  $C^{13}(\alpha, n)O^{16}$  reaction. Nonthermonuclear processes such as electromagnetic acceleration can produce high-energy protons and neutrons for the building of heavy elements. The correlation of such predictions with various observed abundance anomalies in peculiar stars seems to favor the hypothesis that substantial changes in the abundances of the elements may have occurred since the elements were first created.

Approximate orbital elements from coude spectra have been found by Deutsch for the spectroscopic binary  $\alpha$  Equulei ( $P=98^d$ ). Two spectra are visible, of types gF8 and A3. In the photographic region the composite spectrum observed reveals many of the apparently anomalous effects first noted by Struve in the composite spectrum of Capella: suppression of the strongest metallic lines of the later star, and of the Balmer lines of the earlier star; and unusual widening of the metallic lines of the earlier star. It remains to be seen whether these apparent anomalies can be understood in terms of the blending of two normal spectra.

Walker and Osterbrock carried out a search for blue stars in a region in Praesepe. Blue and yellow plates were blinked to pick out stars for photoelectric measurement. Of the stars segregated in this way, five were bright enough to be measured



photoelectrically at Mount Wilson. None of these stars has a particularly blue color in the normal  $B-V$  system; all of them, however, have ultraviolet excesses when compared with the normal near-by stars. Colors and magnitudes of three of the stars are:

| Star    | B     | B-V   | U-B   |
|---------|-------|-------|-------|
| D ..... | 15.74 | +0.59 | +0.02 |
| E ..... | 15.60 | +0.59 | -0.07 |
| Z ..... | 16.25 | +0.47 | -0.25 |

Although it is not certain, it is likely that these are background stars, for if they were in the Praesepe cluster they would have absolute magnitudes of the order of  $M_v=9$ . Some additional blue stars, too faint for photoelectric three-color measurement at Mount Wilson, were also discovered in this survey. One of these fainter stars has been announced as a possible white dwarf by Luyten.

A number of faint ( $m_{pg}=12$ ) red giant stars ( $m_v=10$  to  $12$ ) in the neighborhood

of the north galactic pole have been observed by Münch for spectral classification. Most of the stars observed so far have been found to exhibit the spectral characteristics of near-by giants of types G and K.

In connection with some spectrophotometric work now in progress on two peculiar A stars (HD 125248 and  $\gamma$  Equulei) and a metallic-line star (8 Comae), a theoretical study was made by Deutsch of the effect on derived equivalent widths of finite width of the analyzing slit at the microphotometer. The effect was shown to be one of systematic exaggeration of the equivalent widths, the error amounting to over 30 per cent in the case of deep, narrow absorption lines analyzed with a slit as wide as the resolving limit of the plate. This effect is probably partly responsible for the fact that measures of low-dispersion spectra have often given equivalent widths systematically larger than those found at high dispersion.

## GASEOUS NEBULAE AND INTERSTELLAR MATTER

### INTERNAL MOTIONS

Wilson has continued the investigation of the internal motions in planetary nebulae. To extend these measurements over the whole area of the nebulae, multislits have been constructed for both the 18-inch and 72-inch cameras of the 200-inch coude spectrograph. Spectrograms taken with these multislits readily show the variation of expansion velocity across the image of the nebula, and should provide means for comparisons with assumptions of spherical or nonspherical velocity distributions.

The 72-inch camera multislit has been applied by Münch and Wilson to a study of the turbulent motions in the Orion nebula. It has 31 slits, spaced 1 mm apart, and hence gives simultaneously 31 images of a given line separated by 1.3 seconds of arc in the nebula. This resolution is about as high as seeing limitations will ordinarily permit. Nevertheless, plates already obtained indicate numerous examples of real velocity differences between adjacent slit

images. The dispersion of these spectrograms is 4.5 Å/mm (third-order blue), and the uncertainty in determining the velocity of a line image at an average point appears to be of the order of 0.5 km/sec. Lines useful for measurement on most exposures are  $\lambda_{5007}$ , [O III];  $H\alpha$ ; and  $\lambda_{3727}$ , [O II].

Osterbrock obtained spectra for radial-velocity measurement in the emission nebula IC 405. Spectra of four bright patches give velocities between +10 and +30 km/sec, with no measured velocities as high as +60 km/sec, the radial velocity of AE Aurigae, the exciting star. The interpretation of Blaauw and Morgan that the star and nebula are moving through space together is thus not confirmed by these observations; neither it is ruled out. It is clear that if an irregular cloud moving with the star were colliding with an irregular cloud more or less at rest in space, the regions where material from the two clouds was meeting would be densest

(brightest) and also have the lowest velocities. Further observations are planned to get longer exposures and thus measure the velocities of fainter patches in IC 405.

#### WAVE LENGTHS OF FORBIDDEN LINES

Extensive measurements of a series of long-exposure spectrograms of eight bright planetary nebulae have been made by Bowen for the purpose of obtaining more precise wave lengths of the forbidden lines. Since in general these forbidden lines cannot be observed directly in the laboratory, it has been necessary to calculate their wave lengths from the differences in the wave numbers of lines in the extreme ultraviolet. The laboratory predictions have therefore, in most of the cases, been uncertain by an angstrom or more. The plates for the present investigation were taken at dispersions from 9 to 57 Å/mm using the coude spectrograph of the 200-inch telescope. Where possible, wave lengths were measured in several nebulae to reduce the effects of small velocity differences between the sources of the forbidden lines and of the permitted lines of known wave length. The wave lengths of

about 75 forbidden lines have been determined. The remaining uncertainties in the wave lengths range from 0.01 to 0.02 Å for the bright lines measured on six or more plates of high dispersion, to some tenths of an angstrom for the faintest lines appearing on only one or two of the low-dispersion plates.

#### INTERSTELLAR ABSORPTION LINES

The observation of interstellar absorption lines in very distant stars has been continued by Münch. The stars around  $l=0^\circ$ ,  $b=0^\circ$  recently discovered by Morgan, Whitford, and Code, which are at distances of the order of 6 Kpc, have been observed to have extremely strong and complex interstellar H and K.

In connection with the interpretation of the data being obtained, efforts are being made to establish a general method for constructing curves of growth (line intensity vs. distance) for arbitrarily given velocity distribution of the interstellar clouds. The problem of determining this velocity distribution, under less restrictive conditions than before, is also under study.

### GALAXIES

#### ANDROMEDA NEBULA (MESSIER 31)

One of the first major programs planned for the 200-inch telescope was a redetermination of the distance of the Andromeda nebula. The extensive plate material collected during the year 1950-1951 by Baade provided the data for the study of the variable stars in three special fields of M 31 with centers at  $17'$ ,  $35'$ , and  $50'$  south preceding the nucleus. Work on the light-curves of these variables has been under way since 1952, by Miss Swope on the first and third fields and by Dr. S. Gaposchkin of the Harvard College Observatory on the second. During the past year the investigation of the light-curves of these variables was practically finished, so that data for more than 500 variables are now available. Although the overwhelming

majority are cepheids, the long-period variables and the semiregular and irregular variables are well represented, as are novae and eclipsing systems. Since during the past year Baum's photoelectric sequence in Selected Area 68 became available (it extends to  $m_{pg}=23.2$ ), it was possible to utilize the photographic transfers of SA 68 and to convert all former photometric scale readings into magnitudes on the International System. The resulting new apparent distance modulus for the Andromeda nebula is  $m-M=24.25$ , compared with Hubble's old value  $m-M=22.2$ . The larger part of this difference, 1.5 magnitudes, is due to the recent change of the zero point of the type I cepheids, the remaining 0.7 magnitude to improvement of the photometric scale by photoelectric



measures. That the magnitudes used by Hubble at  $m_{pg}=20.0$  needed a correction of the order of  $+0.63^m$  was already obvious in 1944.

The cepheids in the Andromeda nebula confirm beautifully the earlier conclusion that the spiral arms in such galaxies are regions of high concentration of the dust. The observed period-luminosity relation for the cepheids of such a spiral arm is represented, not by a narrow band, as for instance in the Magellanic Clouds, but by a wide curtain of which only the upper limits are well defined. Even in the relatively "clear" arm  $50'$  south preceding the nucleus, the median magnitudes of the cepheids of a given period scatter over 2 magnitudes.

This scatter due to absorption within a spiral arm should turn out very much smaller in the fourth field of the Andromeda nebula, which is centered on an outlying faint spiral arm  $96'$  south preceding the nucleus. Most of the former dust in this arm seems to have been converted into stars, since distant galaxies shine through in large numbers. Until the cepheid data for this fourth field are available, the distance modulus given above should be considered provisional. An attempt to derive the true distance of the Andromeda nebula from the observed modulus at this time would be premature, since there still exists a large uncertainty about the amount of absorption which the light suffers on its passage from the Andromeda nebula to us, especially within our own Galaxy. Investigations to determine this absorption are under way both here and at the Lick Observatory.

The search for novae in the Andromeda nebula has been continued by Arp through nine months of the 1953-1954 season and two months of the 1954-1955 season. Eighteen novae have been discovered in this period, and at the completion of the program in early 1955, there should be approximately 28 novae for which the brightest three or four magnitudes of the light-curves will have been derived. More

than half of these should have observed maxima.

A network of two-color photoelectric standards is being established over the nebula. These standards, to about the 17th magnitude, will be on varying degrees of background nebulosity, enabling a nova superposed on any degree of background light to be measured against standards on similar background.

Before reduction, the major preliminary results seem to be the large range in apparent maximum magnitude, 1 to 2 mag., and the large range in duration of the novae, from the order of a week to many months.

Baum and Dr. Martin Schwarzschild of Princeton University Observatory have investigated the star-count-to-brightness ratio in several regions of the Andromeda nebula to obtain further information on the stellar content of the nebula.

The count-brightness ratio is the number of resolved stars per unit area divided by the measured surface brightness. It depends critically upon the relative number of stars just below the photographically resolved limit as compared with the number of stars brighter than the limit, and it is a quantity which is sensitive to the difference between population I and population II for a stellar system whose distance modulus is of the same order as the photographic limit of the telescope used. Material obtained with the 200-inch telescope is well suited for examining stellar populations in the Andromeda galaxy and its companion by this method.

Counts of resolved star images were made in certain selected regions, in the outskirts of M 31 and its companion, using fully exposed yellow plates (Eastman 103a-D with a Schott GG-11 filter) obtained during good seeing. By means of the photoelectric photon counter, the surface brightnesses of small patches within these same regions were then compared with one another, and the zero of the surface-brightness scale was based on addi-



tional patches at larger distances from M 31.

The brightest red giants of population II in the elliptical companion were found to be of magnitude 21.2, which corresponds to a modulus of 24.2 if they are identical in absolute magnitude with the red giants in globular clusters. This finding is exceedingly interesting, because it is in perfect agreement with Baade's latest estimate of the modulus. The limit chosen for the star counts was magnitude 22.2, which dips just one magnitude into the population II membership.

The surface brightnesses of the various patches fell in the range from 22.8 to 24.8 photovisual magnitude per square second of arc, and the color indices were all found to be close to +0.77 on the International System. This uniformity of color is puzzling, because some of the patches are primarily population I and others are population II; moreover, the nucleus of M 31 and the nucleus of its companion are both supposed to be population II, but are found to have widely different colors which bracket the color of the patches.

The count-brightness ratios clearly suggest that the regions in the outskirts of M 31 are dominated by population I as defined by stars in the solar neighborhood, whereas those near the companion are in agreement with population II as defined by stars in globular clusters. It follows from this result that the very large masses which elliptical galaxies seem to possess can be explained only by assigning them an enormous number of extremely faint stars (say, below  $M_v = +12$ ) which contribute relatively little light for their mass, or by assuming that the population of the more massive ellipticals may differ from that of the companion to M 31.

#### LOCAL GROUP OF GALAXIES

With the tapering off of the Andromeda program, observing time became available for Baade's long-planned investigation of the resolvable pure type II galaxies of the Local Group. Two groups of objects are

involved: the three Sculptor-type systems in (1950.0)

|               | R.A.            |                 |                 | Dec.       |
|---------------|-----------------|-----------------|-----------------|------------|
| Leo .....     | 11 <sup>h</sup> | 10 <sup>m</sup> | 47 <sup>s</sup> | +22° 26'.0 |
| Ursa Minor .. | 15              | 8               | 15              | +67 23.8   |
| Draco .....   | 17              | 19              | 21              | +57 56.9   |

and NGC 185 and NGC 205. The first three systems are close enough so that with the 200-inch telescope the cluster-type variables can be reached, and the latter have been found in all three of them. But the stellar content of these Sculptor-type systems is so low that as samples of population II they should not provide much information beyond that already obtainable from the globular clusters of our own Galaxy. The very much richer systems NGC 185 and NGC 205 should fill this gap. Although the cluster-type variables in both these galaxies are just beyond the reach of the 200-inch, the investigation of these two systems should furnish much-needed information about the brighter variable stars of population II.

The observations of NGC 185 and NGC 205 were started two years ago by Baade. During the present year the observing program on the three Sculptor-type systems went into operation. Farthest advanced are the observations of the Draco system, for which during the year 130 plates, including those necessary for the color-magnitude diagram, were obtained. The plates, which cover only the central region of this system, were thoroughly searched for variable stars. Two hundred were found, of which the overwhelming majority are cluster-type variables. For the more distant Leo system, the plates cover the whole system, and 160 variables have been found thus far, but the search is far from complete.

A new solution for the solar motion with respect to the Local Group of galaxies has been derived by Humason and H. D. Wahlquist from what are believed to be improved velocities of these objects. Most of the spectroscopic work was done at the prime or coudé focus of the 200-inch and

with higher dispersion than has been used in the past. In all, thirteen objects were observed, of which eight were classed as certain members and five as doubtful members. Lick velocities for the two Magellanic Clouds were also used in obtaining the solution. Eleven solutions were derived from various combinations of the data. A final value was obtained from the mean of what were considered to be the two best solutions. These solutions gave  $292 \pm 32$  km/sec for the apical velocity, and the galactic co-ordinates of the apex were found to be  $l_0 = 74^\circ.6 \pm 6^\circ.3$  and  $b_0 = -6^\circ.8 \pm 4^\circ.2$ . These values are in good agreement with those previously obtained by Hubble and Mayall.

A star cluster, found by Harrington and Zwicky, was analyzed with the 200-inch telescope by Zwicky. This object is at R.A.  $21^h 43^m 49^s$  and Dec.  $-20^\circ 29'$  (1950.0). Its brightest stars are photographically about  $m_p = +16.5$  to  $+17.0$ . Assuming that these stars, which are red, have an absolute photographic magnitude  $-2$  to  $-2.5$ , the distance modulus of the system is between 18.5 and 19.5. Since the integrated apparent magnitude is about  $+15.0$ , the absolute photographic magnitude of the whole system becomes  $M_p = -4$  to  $-5$ . If, as Zwicky believes, this object is a galaxy, it is one of the faintest known. Outside the Local Group a great number of dwarf galaxies have been found which belong to interconnected groups and which are fainter than  $M_p = -10$ .

#### THE MESSIER 81 GROUP OF GALAXIES

The study of the group of galaxies which are centered on the giant Sb M 81 has continued during the report year. The relatively loose cluster is of great importance in the general problem of the redetermination of the cosmic-distance scale, since this group and the group associated with M 101 provide the only galaxies where cepheids can be isolated outside the Local Group. In a very fundamental sense, the Local Group and the M 81 and M 101 groups are

important, since they provide the only systems where direct calibration of the criteria of brightest stars and irregular variables against the cepheids is possible. Hubble's distance scale of 1936 rests entirely on this calibration in only seven objects of the Local Group (our Galaxy, M 31, M 33, NGC 6822, IC 1613, LMC, and SMC). These seven are all that were available before the 200-inch telescope increased the sample to nearly twenty by making it possible to detect cepheids in the M 81 and M 101 groups. Hence, to provide the data necessary for the revision of the fundamental calibrations of the criterion of bright stars, extensive study of M 81 and its companion galaxies has been continued, first by Hubble and later by Sandage. This study has led to a preliminary new distance to M 81, and will eventually lead to the data for the dependence of the absolute luminosity of the brightest resolved stars on content and type of the galaxies.

The M 81 group consists of 9 certain members for which either Humason or Mayall has velocities, and at least 6 probable members which are faint. These faint objects are of the Sculptor and Fornax types and were picked up this year by inspection of schmidt plates in the red. M 81 is the only Sb of the group. There are two Sc galaxies, NGC 2403 and 2976. There are four highly resolved irregulars of the Magellanic Cloud type and two unresolved irregulars whose prototype is M 82. At the end of the report year, 23 normal novae are known in the M 81 galaxy; 3 definite cepheids of periods 30.65, 30.08, and 29 days; 7 irregular bright blue variables of the type found in M 31 and M 33; 1 long-period red variable of period 400 days with  $M_{pg} = -6.6$  (max); and about 15 variables whose nature has not yet been determined. In NGC 2403, 27 variables have been isolated thus far, 10 of which are definitely cepheids whose periods range from 54 to 18 days. Among the others, several have cepheid characteristics but no periods are yet available. No novae have been found in NGC 2403. The com-



parison of the number of novae in M 81 (Sb) and NGC 2403 (Sc) shows the same disparity as that between M 31 and M 33, and probably indicates that novae are very unlikely in Sc galaxies as compared with Sb.

A preliminary distance to M 81 was obtained by Sandage from three independent distance criteria: the cepheids, the brightest irregular variables, and the normal novae. The mean of the three determinations gives  $m-M=27.1$  for M 81, which is 3.1 magnitudes fainter than Hubble's 1936 value of 24.0. This makes the M 81 group more distant, by a factor of 4, than was previously believed, and illustrates the very uncertain situation at present concerning the true correction to the distance scale. Besides the Baade-Blaauw correction of 1.5 mag. to the absolute magnitudes of the cepheids, an additional correction arising from the incorrect apparent-magnitude scales available in 1936 must be applied. These two factors combine to give a final scale correction for objects in the general field which is certainly larger than a factor of 2 and may approach a factor of 4. Continued work in the M 81 group, in the M 101 group, and on out into the general field will be required before the final scale value and hence the value of the Hubble expansion constant can be isolated.

Spectroscopic observations of the nucleus and ten emission patches in the large Sc galaxy NGC 2403 have been obtained by Humason for a determination of the mass and spectroscopic rotation. As the spectra of most of the nebulosities contained many sharp narrow emission lines, the velocities are well determined, many of the points having been observed two or three times.

#### DISTANCES OF GALAXIES BEYOND THE VIRGO CLUSTER

The distances of near-by galaxies out as far as the cluster of galaxies in Virgo can be estimated by the successive application of various distance indicators such as cepheids, novae, and globular clusters. Beyond the Virgo Cluster, cosmic distances

throughout the remaining 99 per cent of the universe must then be deduced by intercomparing the integrated properties of the galaxies themselves, using the Virgo Cluster as a cosmic yardstick. During the past year a long-range photoelectric program was initiated by Baum to carry out this intercomparison of galaxies by using the radial spread of light (specifically, a curve of magnitude versus  $\log r$ ) to characterize each galaxy, instead of using its so-called "total" magnitude, on which exploratory investigations of the relation between red shift and distance have thus far depended. The importance of avoiding "total" magnitudes was emphasized in the last annual report, which described preliminary photoelectric measures of the radial distribution of light in typical near-by galaxies, mentioned the absence of observable boundaries, and noted the critical dependence of the observed magnitude on the degree to which the tenuous but large outer regions of a galaxy are included in the measurement.

The present phase of this photoelectric program includes five clusters of galaxies whose red shifts are distributed over the range now observable. Measures of magnitude versus  $\log r$  using the photon counter at Palomar during the past year included three 19th-magnitude galaxies in the most distant of the five clusters (Cl 0925, +2044  $\Delta\lambda/\lambda=0.19$ ). Owing to their faintness and extremely small apparent spread, these objects required exceptionally fine seeing and the utmost precision of measurement. A series of concentrically positioned pin-holes ranging in diameter from 0.25 to 1.5 mm was used both to photometer the galaxies and to determine corrections for diffraction, scattered light, and seeing. The usual procedure for locating faint objects, by offsetting the photometer from brighter objects in the field, required some refinements for this work.

Before a complete interpretation of the results of this program will be possible, it will also be necessary to measure the spectral energy distributions of galaxies in each



of the clusters selected. This complication emerges from Stebbins and Whitford's discovery that the spectral energy distribution of light from remote galaxies varies with red shift in a different manner from that to be expected if the red shift were the only phenomenon affecting it.

#### DISTRIBUTION OF GALAXIES

Zwicky has continued the investigation of the morphology of individual clusters, that is, the internal distribution of the member galaxies. Clusters were classified as very compact, compact, medium open or loose, and very loose. Numbers of member galaxies in various luminosity ranges have been determined. It is found that on the average for many clusters the luminosity function of cluster galaxies rises exponentially with algebraically increasing values of the absolute photographic or photovisual magnitudes. The large-scale distribution of the centers of clusters was studied. In regions unobscured by either interstellar or intergalactic matter, the distribution of these centers, to the limit of the 48-inch schmidt telescope, was found to be random. Zwicky finds a very pronounced deficiency in the number of distant clusters of galaxies in the lines of sight covered by the Virgo Cluster and the Coma Cluster, which he interprets as indicating the presence of extended intergalactic clouds of obscuring matter within these clusters. Whereas the 18-inch schmidt reaches about one to two large clusters per 100 square degrees, the 48-inch schmidt reaches, as expected, about 50 times as many. Beyond the range of the large schmidt, however, either intergalactic dust or some specific characteristics of the universe enormously deplete the number of theoretically expected clusters, so that with the 200-inch only very disproportionately few additional clusters are found. The study of the number of clusters per unit solid angle and as a function of angular size has been continued. Confirmation has been obtained for the previously reported finding that this number, with decreasing angular size,

does not increase so fast as should be expected in an expanding universe, but that it fits very well the curve derived for a stationary universe except in regions where either intergalactic or interstellar dust interferes. The study of luminous intergalactic matter within clusters has been continued.

An average of 2500 galaxies per square degree have been counted by Zwicky on red plates with the 48-inch schmidt. It has been observed that in regions where the stars are distributed absolutely at random, the dispersion of the galaxies counted increases roughly in proportion to the total number of galaxies. This observation may be explained in terms of the existence of absorbing clouds of intergalactic matter.

#### LUMINOUS INTERGALACTIC MATTER

Three types of formation have been investigated by Zwicky: (1) spirals which are directly interconnected by extended spiral arms; (2) spirals and other types of galaxies which are interconnected by sharp taffy-like filaments having no apparent relation to the spiral arms or other distinct features of the galaxies involved; (3) diffuse clouds of luminous matter in which a number of galaxies are embedded.

One of the most interesting cases is that of the three galaxies IC 3481, Anon, and IC 3483, which appear to be interconnected by luminous filaments and whose apparent velocities of recession, according to Humason, are 7304, 7278, and 108 km/sec, respectively. This group is located within the limits of the Virgo Cluster, and the measured red shift of IC 3483 would indicate that it is a member of that cluster, whereas the measured velocities of the other two galaxies seem to indicate a much greater distance. If, on the other hand, IC 3483 is at the same distance as the other two, as Zwicky believes, then this finding would represent the greatest puzzle in the interpretation of the red shift. The next greatest difference in radial velocity found so far for interconnected galaxies is the one in Stephan's quintet (NGC 7317,

7318a, 7318b, 7319, 7320), which amounts to 1100 km/sec.

#### VELOCITY DISPERSION IN THE NUCLEAR REGION OF GALAXIES

It is well known that the Doppler effect caused by random motions of stars widens the lines in the spectra of galaxies. With the aid of the 8.4-inch camera of the coude spectrograph it has now become possible to investigate the size of these random motions with adequate dispersion (38 Å/mm). In general, the velocity dispersion is so large that very few of the features in the spectrum are even approximately single absorption lines, and most of these are strong lines whose appearance depends on the spectral type. A precise determination of the spectral type thus became an integral part of the investigation. The detailed comparison of a stellar spectrum showing well defined absorption lines with the spectrum of a galaxy showing only diffuse blends of lines presents a difficult problem. This was solved by replacing the regular slit of the spectrograph by diffuse slits consisting of glass plates with a non-uniform deposit of aluminum made in such a way that the transmission varies as a Gauss function. With the aid of diffuse slits of this type with different widths, spectra of standard stars were obtained which show the appearance of spectra of various types with different widening of the absorption lines. By comparison—either on a comparator or on microphotometer tracings—of the spectrum of a galaxy with calibration spectra, spectral type and velocity dispersion can be determined.

Preliminary results for the nuclear regions of the Andromeda nebula (M 31) and its brighter companion M 32 have now been obtained by Minkowski. It was found that the spectral types of both galaxies in the region between  $\lambda 3900$  and  $4400$  are between G8 and K0, distinctly later than the types, G5 for M 31 and G3 for M 32, determined by earlier investigators. A small part of the difference is due to the use of the Morgan-Keenan system in the present

investigation, but the major part results from the difficulty of comparing spectra with sharp and with highly diffuse lines. The new spectral types remove the discordance between the spectral type and the color class, which corresponds to later spectral types than those previously accepted. The luminosity class of the spectra has not yet been definitely established. The spectrum of M 32 seems to correspond to relatively low luminosity; this may help to explain the high ratio of mass to luminosity in elliptical galaxies. Preliminary values of the velocity dispersion are  $\sigma = 100$  km/sec for M 32 and  $\sigma = 225$  km/sec for M 31. Exploratory spectra show that M 81 is very similar to M 31, as might be expected. In the nuclear region of NGC 3115, the velocity dispersion seems to be at least as high as in M 31, if not higher; since low-dispersion spectra suggest relatively sharp lines at a moderate distance from the nucleus, the velocity dispersion in this galaxy may depend strongly on the distance from its center.

#### VELOCITIES OF GALAXIES

The new catalogue of red shifts of galaxies has been nearly completed by Humason. All the observational data have been collected, and the results will be ready for publication by March 1955. The catalogue will contain the red shifts and spectral types of some 600 galaxies.

#### PHOTOMETRY OF GALAXIES

The photoelectric measures of magnitude and color index on the International Scale of 558 individual galaxies and 3 double systems, begun in 1947, were completed by Pettit. These measures are part of the reinvestigation of the velocity-distance function for which Humason and Mayall have obtained the radial velocities. A by-product is the determination of the magnitudes and colors of the central stars of Selected Areas in the  $30^\circ$  zone and an intercomparison of 51 stars of the Polar Sequence.



About half the galaxies were measured with two or more apertures, and for 127 objects an aperture small enough to isolate the nucleus was used. The color indices of the nuclei range from 0.31 to 1.51 mag., and 46 per cent exceed 1 mag. Generally, the nuclei are redder than the whole galaxy, but 12 per cent are bluer.

The magnitude-color diagrams of the galaxies in the E and S classes show definite trends. In the E class the formula  $C.I. = 0.04 (m_{pg} - 9 \text{ mag.}) + 0.84 \text{ mag.}$  applies, whereas in the S class the formula becomes  $C.I. = 0.08 (m_{pg} - 9 \text{ mag.}) + 0.50 \text{ mag.}$  The two classes have about the same

color index at magnitude 17, but for the brighter galaxies the E class is the redder. In type Sb the scattering is too great to show a trend. Only 20 irregular galaxies were measured. No zones of restricted color index were apparent. The survey includes members of most of the clusters of galaxies, for example more than 30 members of the Coma Cluster.

The work on an Office of Naval Research contract for the photometry of all galaxies brighter than the apparent magnitude  $m_p = +15.0$  has been continued by Wild, H. S. Gates, and Zwicky, using the 18-inch schmidt camera.

### RADIO SOURCES

Continued investigation by Baade and Minkowski of the radio source in Cassiopeia emphasizes the unique characteristics of this object. The observations seem to establish with increasing certainty the coexistence of two different types of filaments: diffuse filaments associated with the northern arc of nebulosity, and sharp broken bits of nebulosity scattered over the whole area of the nebula. The diffuse filaments show lines of  $[O\text{ I}]$ ,  $[O\text{ II}]$ ,  $[O\text{ III}]$ ,  $[Ne\text{ III}]$ ,  $[S\text{ II}]$ , and  $[S\text{ III}]$ ; in some of them, the red pair of  $[S\text{ II}]$  is of outstanding intensity; lines of  $H$  are not observed. The sharp filaments show  $H\alpha$ ,  $[N\text{ II}]$ , and faintly  $[O\text{ I}]$ ;  $H\beta$  is not observed and must be at least 20 times fainter than  $H\alpha$ . The interpretation of the observed line intensities meets difficulties which have not yet been fully resolved, but leads to the conclusion that the diffuse filaments have relatively low electron density, about  $10^2$  to  $10^3$ , and electron temperatures of the order of  $20,000^\circ$  or higher. The sharp filaments must have very low electron temperature, as indicated by the steep Balmer gradient. Their density is probably high, but the degree of ionization may be low, so that the electron density is only of the order of  $10^2$  to  $10^3$ .

The two kinds of filaments are similarly distinguished by their internal motions. The observed radial velocities are about

400 km/sec for the sharp filaments, but range from  $-2100$  to  $+4800$  km/sec for the diffuse filaments. These large internal motions in some of the filaments of this nebulosity lead one to expect changes in form and structure within relatively short intervals of time. Comparison of plates taken in 1953 with those taken one and a half years earlier clearly showed such changes in a number of filaments. In addition, they revealed a general outward motion of about  $0''.3$  per year for the filaments belonging to the northern arc of the nebulosity. Both these structural changes and the outward motions are restricted to the diffuse nebulosity forming the northern arc. In contrast, the broken bits of sharp nebulosity which register only in red light show neither perceptible changes in structure nor any signs of proper motion. All together, it looks as if two masses of gas were involved. The observations admit the possibility that the sharp filaments form a system expanding with a radial velocity of the order of 100 km/sec.

The unusually large velocities observed in the strongest known radio source suggest some connection between nonthermal radio emission and random motions. An investigation of the random motions in the Crab nebula and in the filamentary nebula IC 443 has therefore been started. The re-



sults show a strong dependence of the radio emission per unit volume on the random motions; the radio emission seems to increase faster than the square of the mean random velocity.

#### NUCLEUS OF OUR OWN GALAXY

Although previous explorations with infrared plates at the 100-inch and 200-inch telescopes and the 48-inch schmidt had shown that the absorption in front of the very nucleus of our Galaxy is so heavy that the chances to penetrate it by photographic methods are practically nil, an at-

tempt was made by Baade to register photographically the strong radio source which McGee and Bolton of the Sydney Radio-physics Laboratory have recently located at  $l=327^{\circ}9\pm0^{\circ}2$ ,  $b=1^{\circ}0\pm0^{\circ}2$ . A two-hour exposure at the 200-inch telescope on a supersensitized fast IN plate behind a filter cutting off all wave lengths less than 6900 Å failed to register even a trace of the radio source. In fact, the absorption in this region is so heavy that only stars lying in front of the absorbing clouds show up, with no trace of the dense population II which fills the region of the galactic nucleus.

#### NATIONAL GEOGRAPHIC SOCIETY—PALOMAR OBSERVATORY SKY SURVEY

In the period July 1, 1953 to June 30, 1954, 356 fields were photographed by Abell and Harrington on red- and blue-sensitive plates, for the National Geographic Society-Palomar Observatory Sky Survey. Of the 356 pairs of plates obtained, 230 are acceptable for the sky atlas. The highest number of acceptable pairs taken in any previous year is 122, in 1952-

1953. Unusually fine observing conditions and improved quality of the photographic emulsions used are responsible for the good progress made during the past year.

As of June 30, 1954, all but 10 of the 879 fields projected for the survey had been photographed, and acceptable pairs of plates were available for 573 of the fields, or about 65 per cent of the total.

#### GUEST INVESTIGATORS

In addition to the regular staff, a substantial number of guest investigators from other institutions have carried out observations with the aid of the instruments at the observatories. The following problems have been investigated by these guests.

Dr. Helmut Abt of Yerkes Observatory made observations of intrinsic variables with periods between 1 and 2 days. There seem to be two groups of these variables, differentiated primarily by their ranges in light, color, and radial velocity. The variables with small ranges in these quantities (e.g., light-range of 0.4 mag.) may be classic cepheids of short periods; those with large ranges (e.g., light-ranges of 1.5 mag.) may be cluster-type variables with periods greater than 1 day. If this identification is correct, stars of these two groups should have differences in absolute magnitude of more than one magnitude.

A typical star of each group has been observed in order to obtain the luminosity of the star and to identify the groups. The procedure will be to combine radial-velocity-, light-, and color-curves according to the method of Wesselink and Stebbins. The brightest variable of the large-range type is BL Herculis ( $m_{pg}=9.3$  to 10.6); eight good spectrograms of 10 Å/mm dispersion were obtained of this star on four successive nights during July 1953 with the 100-inch coude spectrograph. The brightest of the small-range variables is SU Cassiopeiae ( $m_{pg}=6.2$  to 6.7). Because of its large northern declination ( $68^{\circ}$ ) this star could not be observed with the 100-inch coude. High-dispersion spectrograms were therefore obtained at the Lick Observatory. Photoelectric observations of both stars are being obtained by Robert Hardie of the Lowell Observatory to com-

plete the required data. The analyses of these two stars will also include spectrophotometric studies of their spectra.

Extensive studies of the best of the Moore-Chappell lunar plates made with the 36-inch refractor at Lick Observatory have recently been made by Dr. Dinsmore Alter of the Griffith Observatory and Planetarium. One of these plates, taken at 22.07 days, was unusually sharp and was extremely valuable in many ways. The floor of the crater Ptolemaeus, however, appeared to be partially covered by some sort of cloud or fog. Such a report had been made by amateurs long ago from visual observations. At this phase the sun was low enough so that the floor was already very cold. With the sun just a little higher, on one of F. G. Pease's best plates, taken with the Mount Wilson 100-inch, the fog did not appear. In an attempt to obtain more evidence on this question Dr. Alter has taken a series of lunar plates with the 60-inch telescope, in April, May, and June. In order to emphasize any effects of haze, comparisons are being made between photographs taken on IN emulsions through a pyrex red filter and on either IIaO or Eastman 33 emulsions. Results thus far are inconclusive, and the investigations will be continued.

Observations of the periodic comet Encke (1953f) were made by Dr. Leland E. Cunningham of the University of California in September 1953 with the 100-inch telescope. The comet was recovered farther out in its orbit than ever before. Orbits were calculated for two objects discovered at Palomar, 1953e P/Harrington and 1953g Abell.

Spectroscopic observations of red dwarfs have been made by Dr. G. H. Herbig of Lick Observatory with the coudé spectrograph of the 100-inch telescope in the wavelength region  $\lambda\lambda 5200-6750$ . The dispersion used was 15 Å/mm, furnished by the second order of the 10,000 line per inch grating, together with the 32-inch camera. To date, spectrograms of quality fair to good have been obtained of 25 stars with

spectral types between dK6 and dM5; of these, 8 stars have the  $H\alpha$  line in emission. In all the dMe stars for which adequate plates are available, the bright  $H\alpha$  line is divided by a narrow central reversal, which indicates that cooler material lies above the emitting regions. In addition, 5 dMe stars (BD +43°4305, -8°4352AB, +19°5116A, +20°2465, and CD -31°17815) exhibit a narrow emission feature at the position of the D3 line of  $He\text{ I}$  ( $\lambda 5875$ ). Emission is also present in the sodium D lines of +19°5116A. It is hoped that study of these spectrograms may shed some light on the problem of the difference between M-type dwarfs with and without hydrogen emission, and on the nature of the dMe stars.

Dr. T. S. Jacobsen of the University of Washington continued his observations of cepheid variables during the summer of 1953. The details of the emission features and the interstellar cores of the H and K lines are now under study.

Dr. R. B. Leighton of the California Institute of Technology has continued his program for taking motion pictures of the planets in color at the Cassegrain focus of the 60-inch telescope. The apparatus used has been considerably improved, with the addition of a photoelectric guiding system which holds the image steady throughout each exposure, and a zero-deviation prism which compensates the effects of atmospheric dispersion for large zenith angles. Satisfactory sequences have been obtained on Jupiter and Saturn using the automatic guider, and test exposures have been made on Mars using both the automatic guider and the compensating prism. Both these improvements show considerable promise for significantly increasing the resolution of planetary detail.

The program of studies on the far-infrared solar spectrum sponsored by the McMath-Hulbert Observatory has continued for its fifth year. Mr. Dale Vrabec was the observer from July 1 to September 1, 1953, and Mr. Walter E. Mitchell, Jr., from September 1, 1953 to June 30, 1954.



Dr. O. C. Mohler worked with Vrabec and Mitchell between August 15 and September 15. The program of observations for the present year has been the following: observations to detect either seasonal or short-period variations in the  $N_2O$ ,  $CO_2$ , and  $CO$  in the earth's atmosphere; recording of the infrared helium line; obtaining of drift-curves for the derivation of solar-limb darkening in the  $4.6\text{-}\mu$  region; attempts to improve the resolving power and mechanical precision of the spectrograph.

As part of the work on the infrared helium line, an intensive photographic program using both the 60-foot and 150-foot tower telescopes and their spectrographs was carried out by Vrabec and Mohler during the latter half of August 1953. Starting on July 10, the Snow spectrometer was completely overhauled after its special use in the limb-darkening and solar-intensity program. Refinements of mechanical adjustment and some modifications in the equipment were made by Vrabec and were under development by him until he left the project in September 1953. They have been continued by Mitchell. The definition and resolving power in the 10,000–35,000 Å region were markedly improved as a result of this work; and as a final step, a new grating, no. 75B, was loaned to the project by the Mount Wilson Observatory to replace grating no. 42B, on October 29, 1953.

Work to improve the mechanical and optical properties of the spectrometer was continued until interrupted at the end of December by a forest fire. At this time most of the optical components of the spectrograph were removed from the instrument, so that a completely new installation and adjustment was necessary early in January. By mid-February the performance of the instrument had been so much improved that an average tracing now shows resolving powers of about 100,000 at 13,000 Å. On the occasions when the spectrograph seeing is practically perfect, the resolving power approaches 200,000. The excellence of the present performance

of the spectrometer has made obsolete all the previous observations that required high resolving power. A complete new mapping of the spectrum between 8000 and 25,000 Å is about two-thirds completed.

Most of the observing programs have been continued without interruption during the changes in the spectrometer. Observations were made on 170 days, and a total of 1435 tracings was accumulated during the year. New programs made possible by the improved resolving power are being started. Mohler has completed the measurement and identification of more than 6000 lines shown in the infrared atlas. The decision to include detailed identifications of the water-vapor lines, to be supplied by Dr. W. S. Benedict, has delayed publication of the companion volume to the infrared atlas.

Dr. D. H. McNamara of the University of California has obtained spectrograms of the  $\beta$  Canis Majoris stars  $\sigma$  Scorpii, BW Vulpeculae, and 16 Lacertae in order to study their line-profile changes and radial-velocity variations. The stars  $\sigma$  Scorpii and BW Vulpeculae were observed jointly with Dr. Struve. Spectrograms of the eclipsing binaries TX Ursae Majoris and V805 Aquilae were also obtained in order to determine their velocity-curves.

Dr. B. Y. Mills of the Radiophysics Laboratory of the Commonwealth [of Australia] Scientific and Industrial Research Organization studied the problems of the identification of discrete astronomical sources of radio noise with optically observed objects.

The program of searching for cyclic variations of H and K emission lines in the spectra of dwarf stars has been continued into its fifth year by Dr. Daniel M. Popper of the University of California at Los Angeles. About 170 spectrograms of 7 stars have been obtained without revealing any marked changes of intensity of H and K. However, the two stars with weak hydrogen emission (discovered in this program) have both shown intensity variations of the hydrogen lines. HD 234677 (8.3, Mo)



showed a strong flaring of the hydrogen lines on one out of seventeen spectrograms. No other definite variations are present. HD 224085 (7.3, K2) shows slight but almost certain variation of the intensity of  $H\epsilon$  ( $H\delta$  and  $H\gamma$  are swamped by the continuum) on the six 100-inch spectrograms. Variations are suspected also on the seven 60-inch plates, but the resolution is not quite sufficient. Thus it appears that if cyclic variations are to be found by this method, stars with  $H$  emission are the most promising, and that HD 224085 is the best candidate to date.

The most important new result obtained in the program of eclipsing binaries with components above the main sequence is that RZ Cancri shows double lines on coudé plates of the visual region. Previous work on this system of two late-type giants had failed to show duplicity. Its analysis should approximately double our knowledge of the masses of late-type giants. Work on other systems of this class, reported on a year ago, is continuing. Dr. Popper is also carrying on to a limited extent the earlier program of investigating eclipsing binaries with marked discrepancies between spectrographic and photometric data. In this program a series of spectrograms of the short-period A-type system S Antliae, of quality much superior to that of the older ones of thirty or more years ago, has failed to show lines of the secondary component. This result, if verified, would remove one outstanding discrepancy.

Spectrograms of 40 Eridani B, taken the preceding year by Dr. Popper, yield a red shift (the effect of motion having been eliminated) of  $21 \pm 4$  km/sec as compared with the value  $17 \pm 3$  predicted from the mass and radius by the theory of general relativity.

Dr. M. Schwarzschild of Princeton University Observatory, assisted by Mr. J. Rogerson and Mr. A. Skumanich, investigated the turbulence spectrum of the solar atmosphere. The measurements were based on direct solar photographs and spec-

troheliograms taken at the 60-foot tower by Hickox. The turbulence spectra were derived for the intensity in  $H\alpha$ , in the K line, and—in collaboration with Dr. F. N. Frenkiel—in the continuum. The statistical symmetry of dark and bright elements in the granulation was investigated and a high degree of symmetry found.

Dr. Schwarzschild took high-dispersion spectra of five additional K giants with the 100-inch coudé spectrograph to enlarge the earlier program of comparing high- and low-velocity stars.

Baum has measured the surface brightness of selected fields in the Andromeda nebula and its elliptical companion NGC 205, and Dr. and Mrs. B. Schwarzschild have counted the resolved stars in the same fields on plates taken by Baade. The count-brightness ratio thus derived and the mass-luminosity ratio compiled from other sources form the basis of a discussion of the stellar content of these systems.

Roger Tayler, also of Princeton University, has derived the luminosity function of Messier 92 from counts on plates taken by Sandage. The theoretical analysis of the results indicates an initial luminosity function for globular clusters with a very steep drop at about absolute magnitude +2.

Dr. F. Graham Smith of the Cavendish Laboratory of Cambridge University studied plates of the National Geographic Society-Palomar Observatory Sky Survey to correlate radio observations of discrete astronomical sources with objects recorded optically on plates of this survey. He also made an attempt to establish a receiver for the study of ionospheric twinkling, by means of the disturbances of the interference pattern of bright discrete sources produced by random clouds of ions. This apparatus was set up at Palomar Mountain, and although no definitive results were obtained, it represented the first radio-astronomy experiment at these Observatories.

Professor John Strong and Dr. William Sinton of Johns Hopkins University made a study of the temperature and spectrum

of Venus, using the 200-inch telescope at the coudé focus. They used a double monochromator to isolate a band of radiation from Venus extending from 8 to 14  $\mu$ , which was used to determine the temperature. This monochromator was also used to get narrow bands (about  $\Delta\lambda=0.3 \mu$  wide) to determine the spectral components of the radiation.

The planet was approximately in dichotomy. Scans were made parallel and perpendicular to the terminator to get the distribution of temperature. This temperature averaged 235° K, with a range of only 5° along the "equator" from the noon limb to the midnight limb. Along the terminator the temperature was slightly lower at the N and S terminators. The temperature distribution confirms the earlier work of Nicholson and Pettit.

The spectrum, reduced to outside the atmosphere, exhibited weak broad bands which have not yet been interpreted. Additional measures, with Venus as an evening star, will be made during the forthcoming season to complete the study.

An extensive program for the spectroscopic study of the components of visual double stars has been carried out by Dr. Otto Struve of the University of California. During the past year approximately twenty systems have been observed, including a few having separations of the order of one second of arc, or slightly less. The principal result of this work was to bring out the tendency of the more luminous components to have later spectral types than would be consistent with their luminosities. In other words, these brighter components may either be main-sequence stars, or they may be subgiants with different degrees of departure from the main sequence. Undoubtedly these subgiant components represent old stars. Their space motions would, however, not justify regarding them as members of population II; instead, they probably should be regarded as old stars of population I. Among the components of spectral class earlier than about G0 there is a very striking relation between

the amount of rotational line broadening and the luminosity of the component. This relation has the same sense as among single stars of spectral classes B, A, and F. It seems, however, to be obeyed much more rigorously by the double-star components.

Considerable attention has also been devoted to the study of the variable stars of the  $\beta$  Canis Majoris type. A large amount of spectrographic material has been obtained with the 100-inch telescope and the coudé spectrograph for the study of the variations in the line contours of  $\sigma$  Scorpii and BW Vulpeculae. Both these stars show very broad lines on the descending branch of the velocity-curve and relatively sharp lines on the ascending branch. The spectrophotometric study of the line contours is now in progress. Several series of high-dispersion spectrograms have also been obtained for  $\gamma$  Pegasi and  $\xi^1$  Canis Majoris. These two stars have recently been discovered by Dr. D. McNamara to belong to the group of  $\beta$  Canis Majoris variables. In co-operation with Dr. A. van Hoof in Louvain, Belgium, Dr. Struve has investigated differential motions in the atmospheres of  $\beta$  Canis Majoris and 16 Lacertae, as obtained from measurements of different spectral lines.

Considerable attention has been given to the study of effects of large-eddy turbulence in the atmospheres of supergiant stars such as  $\rho$  Leonis,  $\alpha$  Cygni, and  $\delta$  Canis Majoris, and also in the variable stars of the  $\beta$  Canis Majoris type. In this work Dr. S. S. Huang has co-operated with Dr. Struve.

Dr. H. C. van de Hulst of the Observatory of Leiden made a study of the emission hydrogen regions in the Milky Way at the request of the Commission on Interstellar Matter of the I.A.U. Using plates of the National Geographic Society-Palomar Observatory Sky Survey, he constructed a detailed map of these emission regions over a limited section of the Milky Way in an effort to determine the best method of cataloguing these features.

Dr. Uco van Wijk of the Princeton Uni-



versity Observatory has carried out an extensive investigation of the galactic cluster NGC 1746, in the course of which magnitudes of the stars were measured in three colors. A previous study of this galactic cluster by Cuffey had shown that the color-magnitude array is of an unusual type. The photographic observations were made with the 60-inch telescope at Mount Wilson Observatory, using a diaphragm of 32-inch diameter in order to obtain a larger useful field. The ultraviolet magnitudes were obtained with 103a-O plates and a Corning 9863 filter, the blue magnitudes with 103a-O plates and a GG 13 filter, and the yellow magnitudes with 103a-E plates and a GG 11 filter. Stars down to the 15th magnitude were measured in the cluster and in a comparison field one degree west of the cluster. Photoelectric measurements were made for the purpose of calibrating the photographic magnitudes, but this latter part of the program was not completed.

Dr. Harold Weaver of the University of California has used the coudé spectrograph of the 100-inch telescope to obtain spectra of a number of brighter stars in the Hyades. The plates were taken for a two-fold purpose: first, to examine the spectra of giants of early type; second, to compare in detail the spectra of early-type giants and incipient metallic-line stars. Spectra

of several standard stars in the Morgan-Keenan-Kellman classification system were also photographed.

Dr. A. E. Whitford of Washburn Observatory, University of Wisconsin, began a systematic program of photoelectric scanning of stellar spectra during the summer of 1953. About twenty representative stars of various spectral types and luminosities were recorded over the range from 3400 to 10,000 Å. A few reddened stars were included.

The first application of these results was to the calculation of a model atmosphere for  $\sigma$  Bootis, an F2 dwarf. It was carried out by Kenneth Hallam and Ross Douglas, students of Dr. A. D. Code at the University of Wisconsin. The best fit was obtained for an effective temperature of 6760° K and an electron pressure of 56 dynes. The transformation from relative to absolute energy units was done by combining various existing calibrations of Vega. This method gave energy-curves for dwarf G stars in fairly good agreement with that of the sun.

Direct absolute energy calibration of the photoelectric measurements by means of a thermocouple, using artificial sources, was successfully tested at the end of the 1953 season. Certain modifications will be necessary before definitive results can be obtained.

## THE LIBRARY

More than a hundred volumes—mainly books important for the history and philosophy of science—have been received by bequest from Dr. Hubble's library; this gift includes 14 volumes printed in the sixteenth century, 20 in the seventeenth, and 23 in the eighteenth. Special bookplates identify these volumes, now shelved in the Hale Room. There are, among the Hubble copies, a few duplicates of the library holdings, e.g., the *Selenographia* of Hevelius (Danzig, 1647), Riccioli's *Almagestum novum* (Bologna, 1651), and the third edition of Newton's *Principia* (London, 1726). Some of the Hubble

copies bear either the signature or the bookplate of J. L. E. Dreyer; they form a welcome addition to the library's collection of books which used to belong to Dreyer. Kepler's *Epitome astronomiae copernicanae* (Linz, 1618 and 1622; Frankfurt, 1621) offers an example of dovetailing of gifts: the library has the Hale copy, books 1-3, pages 1-418, forming the self-contained "Doctrina sphaerica" of the *Epitome*; the Hubble copy, consisting of books 4-7, pages 419-932, forming the self-contained "Doctrina theorica," completes the *Epitome*.

The Copernicus-Vesalius year, 1543, is



represented in the Hubble gift by three well printed volumes from the presses of three countries: Pliny's *Historia mundi* (Paris, Petit); the *Epitome in Cl. Ptolemaei Magnam compositionem*, by Regiomontanus and Peurbach (Basel, Henricpetri), from the library of the Riccati family; and Sacrobosco's *De sphaera* and *Computus ecclesiasticus* (Wittenberg, Seitz), with prefaces by Melanchthon, whose name was systematically deleted by a sixteenth-century owner. Other sixteenth-century volumes include: Pierre Duhem's copy of Alfraganus and Albategnius (Nuremberg, 1537); a well preserved copy of the Spanish translation of Apian's *Cosmographia* (Antwerp, 1548), with all the moving parts of all the volvelles; Peucer's *Elementa doctrinae de circulis coelestibus* (Wittenberg, 1563), with Dreyer's manuscript warning concerning the brittle paper; the second edition of Copernicus' *De revolutionibus* (Basel, 1566), with anti-heliocentric annotations in a contemporary hand; Magini's pro-heliocentric *Novae coelestium orbium theoricæ* (Venice, 1589).

Two important additions to the library's collection of editions of Galileo's works are: the *Systema cosmicum*, the second

edition (Lyon, 1641) of Bernegger's Latin translation of the "Dialogo . . . sopra i due massimi sistemi del mondo"; and Thomas Salusbury's *Mathematical Collections and Translations*, the first tome (London, 1661), which includes the translation of "Galilaeus . . . His Systeme of the World." Other seventeenth-century works include: Dreyer's copy of Boulliau's *Astronomia philolaica* (Paris, 1645), with the bookplate of Trinity College, Cambridge; Fontana's *Novae coelestium terrestriumque rerum observationes* (Naples, 1646); Sherburne's *The Sphere of Marcus Manilius made an English Poem* (London, 1675); Viviani's *Formazione e misura di tutti i cieli* (Florence, 1692).

The number of bound volumes in the library now exceeds 19,000. The following tabulation shows the growth of the library:

| Date                  | Number of bound volumes |
|-----------------------|-------------------------|
| 1910 March . . . . .  | 1,000                   |
| 1917 August . . . . . | 5,000                   |
| 1928 July . . . . .   | 10,000                  |
| 1939 May . . . . .    | 14,000                  |
| 1947 May . . . . .    | 16,000                  |
| 1954 April . . . . .  | 19,000                  |

## STAFF AND ORGANIZATION

Dr. Edwin P. Hubble died very suddenly on September 28, 1953, thereby bringing to a close one of the most distinguished careers at the Observatories. Dr. Hubble joined the staff of the Mount Wilson Observatory in 1919. Within ten years his observations had revolutionized the concepts of astronomers as to the size and content of the universe.

Before 1920, astronomical measurements of distance had been limited to objects in the Milky Way system, and the universe was believed to consist of this one great disklike system of stars, having a diameter of about 100,000 light-years. Hubble, using the recently completed 100-inch telescope, was able to resolve a representative group

of spiral galaxies into stars and to identify some of these stars in terms of known stellar types. By comparing the apparent brightness of these stars with their absolute brightness as determined from samples in the Milky Way, he obtained a measurement of the distances of the stars and therefore of the distances of the objects in which they were found. These distances at once indicated that these galaxies have dimensions and total luminosities comparable to those of our own Milky Way system. It was evident from these and Hubble's further investigations that the universe consists not of the single stellar system of the Milky Way, but of some hundreds of millions of such systems, extending at least

out to the extreme range of the largest telescopes, a billion or more light-years away.

In succeeding years Hubble and the department which he built carried out extensive studies of the distances, luminosities, masses, structures, and motions of these stellar systems, which he named extragalactic nebulae. Spectroscopic observations, largely by Humason, showed that the galaxies are receding from us with velocities proportional to their distances. This led Hubble to the concept of the expanding universe. These investigations of galaxies, many of which required the full light-gathering power of the 100-inch telescope, emphasized the need of still greater telescopic power and provided one of the chief reasons for the construction of the 200-inch Hale telescope on Palomar Mountain. Hubble actively participated in the design of this instrument and made the first observations with it. Hubble served on the Observatory Committee from the start of the joint operation of the Mount Wilson and Palomar Observatories until the time of his death, and was chairman of the committee on research programs.

Dr. Donald E. Osterbrock joined the staff of the Observatories on July 1, 1953, and plans to undertake a nebular program.

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## COMMITTEE ON ELECTRONIC IMAGE CONVERTERS FOR TELESCOPES

CO-OPERATIVE PROJECT OF MOUNT WILSON AND PALOMAR OBSERVATORIES, DEPARTMENT OF  
TERRESTRIAL MAGNETISM, NATIONAL BUREAU OF STANDARDS, UNITED STATES NAVAL  
OBSERVATORY, AND CALIFORNIA INSTITUTE OF TECHNOLOGY

JOHN S. HALL, *United States Naval Observatory*

### STATEMENT OF THE PROBLEM

The image-converter project was initiated to explore the possible use of electronic techniques, supplanting or supplementing present photographic methods, to increase the range of telescopes.

One limitation of the photographic method lies in the inability of the photographic emulsion to store useful information beyond a definite density which is fixed (for a given emulsion) by the ever present glow of the night sky and by the f-ratio of the telescope. For 103a-O plates, this plate density is reached in 30 minutes with the 200-inch Hale telescope and in 12 minutes with the 48-inch schmidt. Exposures beyond these limits are likely to result in a loss of useful information.

Even if the night sky glow were absent, the photographic method suffers from the failure of the reciprocity law at very low light levels, low quantum efficiency, and nonlinear response. The outstanding advantage of photography lies in its high resolution.

A photoelectric method is now being used extensively on a point-to-point basis, single objects such as stars, nebulae, or asteroids being measured one at a time. An average of one in every five photons can be effectively counted in this way, no saturation exists, and the light intensity measured has a mean error which is inversely proportional to the square root of the number of counts made in a given unit of time. For faint objects the near-by sky must also be measured after the object under observation is removed from the pin-hole at the focus of the telescope. As fainter and fainter stars are observed, the

star contributes a smaller and smaller fraction of the total light.

These conditions affect in extreme degree the measurements being carried on by Dr. W. A. Baum with the 200-inch Hale telescope on Palomar Mountain. The following paragraph, from Baum's report at the Flagstaff Photoelectric Conference of September 1953, describes the problem concisely.

For a 23rd magnitude star measured through a 0.4-mm diaphragm ( $\approx 4''$ ) and a blue filter, the instrument as presently adjusted, registers about 40 counts per second due to night sky radiation, 4 per second due to dark emission and 1 per second from the star. Each observation consists of a series of alternate 100-second counts on sky and on star-plus-sky until the total accumulated time is sufficient to yield the statistical accuracy sought; at 23rd magnitude this process goes on for hours. Not only must one depend upon photographs to show where photometerable stars are located, but one is also at the mercy of background objects which are a little too faint to be revealed photographically. Such objects can introduce serious errors into the measurement of resolved stars.

Because of blurring of the stellar image by inhomogeneities in the earth's atmosphere, stellar images 0.25 mm in diameter are average, and a 0.1-mm image is obtained on only about one night in four. Stars of the twenty-third magnitude represent the limit of distinct photographic detectability. These facts emphasize the need for improving the discrimination of faint images against the sky background if we are to go beyond the present limits of observation with the most powerful telescope.



## RELATED PROJECTS

The two-dimensional problem, in which an area is reproduced by an image converter, has been successfully solved in a restricted sense by Dr. A. Lallemand, of the Paris Observatory. A semitransparent photoemissive cathode is deposited on one side of a glass plate mounted inside a large vacuum system. When an optical image is focused on the photocathode, the electrons ejected by the light are accelerated and focused on a photographic plate inside the same vacuum in such a way that the original image is reproduced by the action of the high-energy electrons on the photographic emulsion. Lallemand has found that a single electron accelerated by 30 kv will produce about ten grains in the emulsion. The high quantum efficiency of the photocathode is fully realized, and the number of grains is to a limited extent proportional to the intensity of the incident light. A serious technical disadvantage results from the necessity of using a different photocathode for each exposure because each plate must be inserted into the system and poisons the photocathode as the system is evacuated. Although Lallemand has obtained a remarkable gain of fifty-fold in sensitivity, his method does not show promise for the solution of the contrast problem, which is so important if present telescopes are to overcome the glow of the night sky and penetrate to greater distances.

Dr. P. B. Fellgett of the Cambridge Observatory has obtained a good image of Saturn in his experiments with an Image Orthicon. His results are to be published in a forthcoming book by F. J. M. Stratton.

Dr. W. A. Hiltner at the Yerkes Observatory reported on his work with image tubes for astronomical purposes at the Flagstaff Photoelectric Conference. He has confined his attention primarily to the problem of achieving the high quantum efficiency of photoelectric surfaces for astronomical applications in which sky background is unimportant.

## MODUS OPERANDI

In February 1954 the Carnegie Corporation of New York awarded a grant of \$50,000 to the Carnegie Institution of Washington for an exploratory program for the development of image converters to increase the range of large telescopes. A committee set up by Dr. Vannevar Bush for this purpose, under the chairmanship of Dr. M. A. Tuve, also includes the following members: Dr. W. A. Baum, Mount Wilson and Palomar Observatories; Dr. L. L. Marton, National Bureau of Standards; Dr. John S. Hall, U. S. Naval Observatory. The initial experiments are being carried out at the Bureau of Standards by Drs. L. L. Marton, E. S. Dayhoff, and R. C. Placious, and the initial tests are to be made at the Naval Observatory by Drs. J. S. Hall and Stewart Sharpless.

The following fundamental data were mutually agreed on by members of the committee as the basis for action:

Elemental area, 0.1 mm square. It is desirable to resolve an area as small as this.

Photon count on elemental area, 300 per second at prime focus of 200-inch mirror,  $f/3.7$ , 1000Å region of spectrum, for a star of magnitude 22.4 at 7000° K. The sky background alone produces the same count for an elemental area.

Photon shower (star or sky) completely random.

Dark emission from an element,  $10^{-2}$  electrons per second at 70° F.

Although these data refer to CsSb surfaces, it would be more desirable to use red-sensitive receivers if such were available, in order to observe the more distant nebulae and other red objects. For the 200-inch application, small size and weight are desirable features. It was agreed, however, that the development of a highly successful image tube should not be inhibited by mechanical limitations.

The work thus far has been mostly of an exploratory nature. One or more members of the committee have visited the

Radio Corporation of America, both at Princeton and at the Lancaster plant. Baum has visited Yerkes Observatory and learned of experiments being carried out there by Hiltner. Marton has visited both British and continental laboratories which are carrying on work pertinent to this project. Professor Lallemand gave the benefit of his experience in this field to members of the committee during a visit to the Bureau of Standards and to Pasadena during May 1954. Dayhoff has made an extensive search of the literature.

#### PROMISING APPROACHES

One of the most promising solutions to the problem was originally proposed to Baum by J. D. McGee of E.M.I. Research Laboratories, in England. McGee's method would include a photoemissive cathode and an image storage system which could be read off in a dark room an hour or so after the exposure at the telescope is completed. Such a tube has never been constructed. In view of McGee's keen interest and broad background in various aspects of this problem, he has been invited to act in an advisory capacity, and has graciously acceded to the request.

The possibility of using commercial television pickup tubes or parts of commercial tubes is being given serious consideration. One possibility is the selenium Vidicon. It has excellent storage characteristics and high resolution. It is likely to develop spots, and requires about ten times as much light intensity as the Image Orthicon in television applications. A second possibility is the Image Orthicon. As normally used, this tube has a storage time of only a fraction of a second; RCA has found that this time may be considerably increased by cooling. The large spot size, lateral conductivity of the storage surface, and poor modulation are the most serious troubles which must be eliminated before a successful tube is made.

Another promising possibility is an experimental intensifier tube recently de-

scribed in a letter to Dr. Bush by Dr. G. A. Morton of the RCA Laboratories. A photoemissive cathode is followed by two intensifier screens and a target identical with that in a television pickup tube. Electrons successively focus the photocathode on these three elements. Since each intensifier screen yields 10 secondary electrons for each incident electron, a hundred-fold gain is achieved within the tube, and there is little or no loss in the process of extracting the signal; only the inevitable photoelectric shot noise remains. The frame repetition rate is 30 per second; Morton suggests that some sort of storage tube might be used for integrating the picture for a longer period of time without loss in efficiency. The resolution corresponds to that of a commercial 400- or 450-line television picture.

Dayhoff has made up circuits for testing Vidicons and Image Orthicons. RCA has kindly loaned samples of these tubes for test at the Bureau of Standards.

#### A CONTINGENT PROBLEM

Since the nature of this project has been made public, astronomers have shown increasing interest not only with regard to the prime problem of probing the universe, but particularly in connection with spectroscopic work. For this problem, high photoelectric efficiency and linear response are of first importance; in only a limited number of problems does sky background inhibit astronomical spectroscopy.

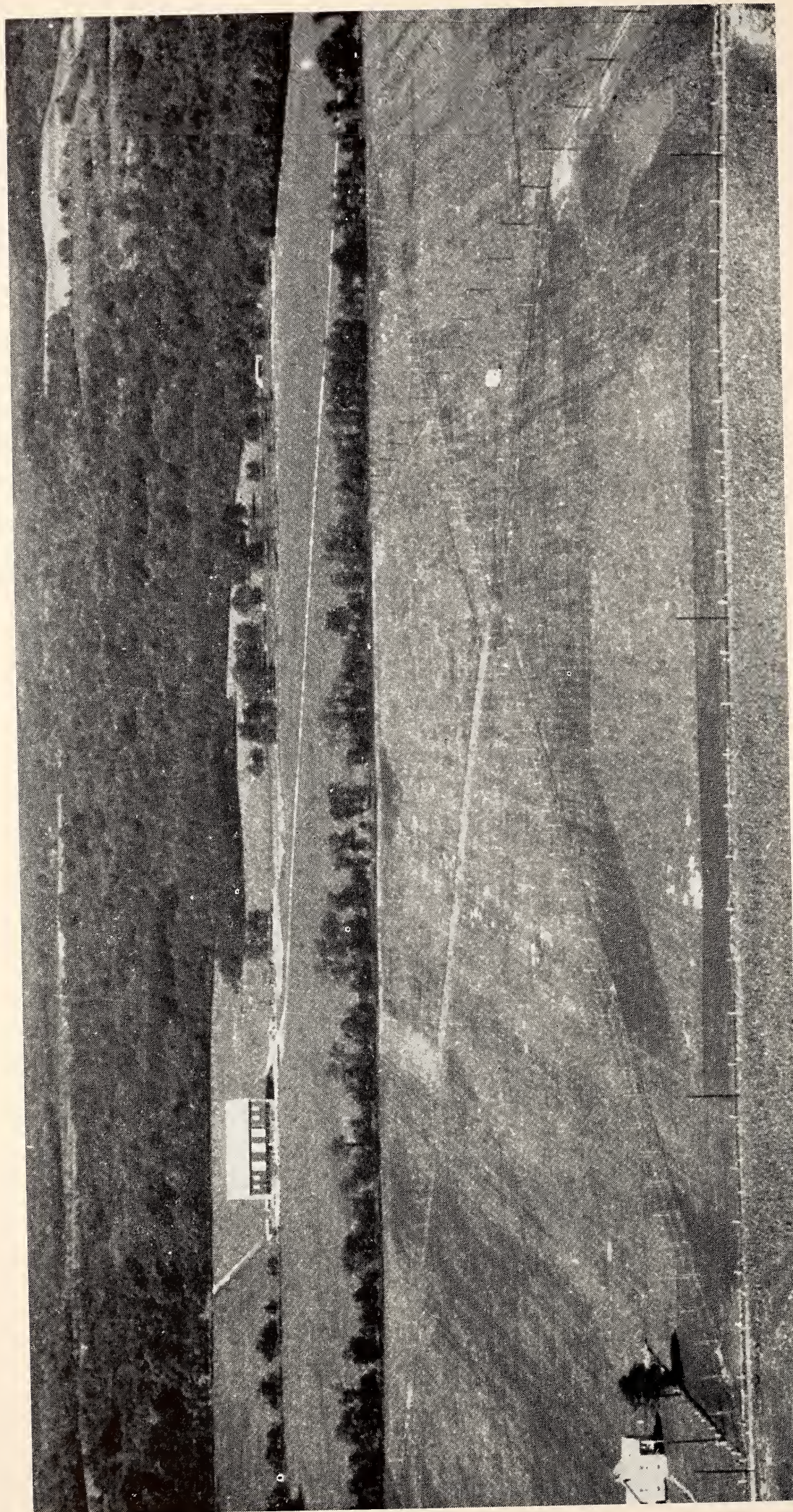
We have mentioned earlier the point-to-point photometry being carried on by Baum with the 200-inch and the great need for a two-dimensional picture with full statistical contrast, which is the ultimate goal of this project. The possibility of setting up a parallel project to seek a solution applicable to the one-dimensional domain of the spectroscopist has also been under consideration by the committee from the start, and activities in this direction are currently being initiated.











*Winged Camera Service*

"Mills cross" antenna for radio astronomy, 22 megacycles. This new antenna is of the crossed-interferometer type. The intersection of two fan-shaped beams defines a small area of sky in which the antenna is receptive to extraterrestrial radio waves. The rows of dipoles are connected through coaxial cables, extra lengths of cable being inserted when the beam direction is to be changed. Each of the two rows is 2048 feet long, and together they occupy the entire 90-acre field in the foreground.



## DEPARTMENT OF TERRESTRIAL MAGNETISM

*Washington, District of Columbia*

MERLE A. TUVE, *Director*

The researches currently in progress in this laboratory are the expression of scientific curiosity and personal interest among a group of well trained men who have had wide experience in modern physics. They share with one another the conviction that physics is properly concerned with studies of process and order and natural law throughout the physical world; the current popular impression that physics is chiefly concerned with atomic energy or the development of new weapons is a gross distortion. The traditional range of interests for physicists, from the astronomical universe to the interior of the atom, is reflected in this report of the year's activities, and the unusual venture of several physicists devoting their efforts to the analytical study of biological processes using the tools of physics is an experiment which continues to bear fruit.

The problem of guiding and encouraging the research activities of a group of investigators in this Institution differs greatly from the same task in a government or industrial laboratory. In our Department it is closely related in spirit and motivation to the support of research in the physics department of a university. Clearly it is not enough for the staff members to keep themselves busy making an extensive series of measurements in some area related to physics or physical laws. It is reasonably obvious that Mr. Carnegie did not set up the Institution simply to keep a dozen men hard at work making measurements eight hours a day in each of several locations. On the contrary, he clearly dedicated these funds to the purpose of freeing certain selected individuals from the necessity of working for a living. He did this just so they would be able to spend their energies on "investigation, research, and discovery," using the facilities also provided by his generous endowment.

This is, then, a creative invitation, not a task assignment. Accordingly, the report of the year's activities which follows below does not aim to demonstrate how hard the individuals in the group have been at work during the year. It is a simplified record of the many ways in which the staff members of the Department have embraced the opportunity to spend their time and energies on problems in physics and related fields which challenge their interest and invite the creative expression of their various technical capacities. "This is not a job we do, but a life we live together" expresses the sense of sharing experiences in explorations of new territory which ties the staff together in the success or failure of their individual projects. The freedom provided by Mr. Carnegie for these selected individuals to devote their time to scientific questions of the highest personal interest, almost literally equivalent to subsidizing the truly "amateur" interests which have evolved out of their advanced training and their research experiences in physics, is the feature which we recognize here as most nearly characterizing the basic purposes of the Institution.

The current activities in radio astronomy, involving studies of radio "noise" emission and absorption by the sun, and by a variety of other astronomical objects, provide an example of the way in which several staff members, new and old, join with spontaneity in exploring the range of opportunity in a fresh subject. Evolving naturally from our decades of research activity concerning the upper atmosphere, the study of these weak emissions from celestial objects now goes far beyond our initial concern with the scintillations in position and brightness of the "radio stars" caused by the changing refraction of the ionosphere. The basic theoretical difficulty that no suitable mechanism has yet been



conceived which might permit clouds of swirling hot gases in or near a star to emit radio waves of the frequencies and intensities which are observed is a challenge to electromagnetic theory and to our knowledge of basic physical laws. The solution may lie in the special properties of ionized gases at very low pressures in magnetic fields.

These studies utilize several radically different arrays of equipment of considerable linear dimensions on three sites near Washington, and the observations concern sunspots, the quiet sun, hydrogen clouds, the gases between the stars, and the energy spectrum of various radio objects, some of them far beyond the limits of our own Galaxy. Similar activities are in progress at only a few other laboratories in the United States, England, and Australia, and our efforts will help to delineate the astrophysical problems which are involved and the several kinds of large-scale equipment needed for their solution.

Another example of a "freewheeling" activity which seeks to open up a whole range of new specialized information, in this case using physics to contribute to geology, lies in the work of the half dozen men here who concern themselves with the earth's crust. Their seminars cover every topic of the physics of the earth, and they make their own rock collections and field observations under the eyes of trained geologists, but their laboratory activities are focused in two technical areas, namely, the use of seismic waves from explosions to study continental structure, including critical analysis of the seismic technique itself by theory and experiment, and the use of microchemical procedures for the dating of ancient rocks, especially igneous intrusives, by isotope measurements on several radioactive elements in separated minerals. The old method of dating by the uranium-to-lead ratios in ore bodies is here vigorously and critically supplemented by studies of common elements, including potassium and rubidium, which are faintly radioactive. Difficulties and contradictions

have naturally beset the earlier years of this ambitious project, and during the current year some of these problems have begun to be resolved. There is reason to hope that these procedures will eventually provide a sound basis for a whole range of new kinds of information concerning the Pre-Cambrian period.

It has seemingly never been considered the business of physicists to be concerned with living matter. Yet living matter is a most conspicuous part of the physical world, and the laws governing process and order in living matter must somehow be an expression of the use of physical laws by the living entity. No position needs to be taken with regard to the question of any additional "vital force" or other non-physical properties of living matter in making the firm statement that the capacity of a living thing to "organize" the nonliving parts of its environment into special chemical entities of higher free energy is worthy of detailed study and contemplation by men trained in physics and interested in the impressive generality or range of applicability of physical laws. In our laboratory, several men with wide training and research experience in modern quantum physics are devoting their thoughts and energies to studies of the synthesis of biological compounds by living organisms. They do not operate here under the guidance or control of biologists or medical men. They select their own problems, and study them in their own quantitative way. Their papers are presented, however, before the biological societies, and guest biologists work in our laboratory on similar problems. During previous years the mechanisms and pathways for the formation of amino acids have been under quantitative examination. These studies are currently being extended to include detailed examination of selected aspects of protein synthesis and the formation of nucleic acids.

Nuclear physics can hardly be ignored by our staff members, most of whom had

their physics training primarily in that field. Their contact with the subject is maintained by the vigorous activities of two staff members who devote all their time to nuclear physics problems utilizing the two high-voltage generators and the cyclotron which are included in the Department's research equipment. During the past year or so, the primary activity

has been concerned with the excitation of various nuclear isotopes to low-lying levels, under one million volts, by the near-by passage of a high-speed electrified particle, usually a helium nucleus. This is called "Coulomb excitation," and the results are of much interest in connection with the collective model and other models of nuclear structure.

## EXPERIMENTAL GEOPHYSICS

### RADIO ASTRONOMY

B. F. BURKE, J. W. FIROR, H. L. HELFER, B. Y. MILLS, L. OWREN, F. G. SMITH, H. E. TATEL, M. A. TUVE, AND H. W. WELLS

Investigations in radio astronomy, as a new direction in the evolution of our upper-atmosphere studies, were continued throughout the year (see Year Book No. 52, pp. 99-102).

The Department was represented at numerous conferences on radio astronomy, including the A.A.A.S. Symposium on "Current progress in radio astronomy" in Boston, December 26-27, 1953. Another conference on radio astronomy was held in Washington, January 4-6, 1954, jointly sponsored by the National Science Foundation, the California Institute of Technology, and the Carnegie Institution of Washington. This conference was called to bring together not only those actively working in radio astronomy, but also a group of other astronomers, physicists, and electronics men, and a number of advanced graduate students and young research men who might undertake work in this area if they were more fully acquainted with its challenges and opportunities. An effort was made to present a comprehensive survey of the present status of research in this field, to examine in some detail the most critical problems now evident, and to indicate, as far as possible, some of the directions for profitable future activities.

### OCCULTATION OF TAURUS

About mid-June of each year the radio star Taurus A, the Crab nebula, is occulted

by the solar corona. When the sun is free of disturbance, the opportunity is presented to observe the effect of the solar corona on the signals from Taurus. June 1954 was probably the last opportunity to observe an occultation of Taurus by the corona of the quiet sun for another decade.

This occultation (see fig. 1) by the solar corona was observed in June 1954 at three frequencies, 22.2, 38.7, and 207 mc/sec. Previous observation by Ryle and Smith at the Cavendish Laboratory on frequencies of 81.5 and 37.8 mc/sec had shown that, instead of the expected refractive effects at a few solar radii, effects were observed at distances of more than 20 solar radii that could only be interpreted as scattering phenomena, thus indicating that the corona is much more extensive than was previously thought. The apparent angular size of the source increased as it approached the sun, an indication that the radio waves were being scattered by condensations of ionized material in the outer corona. These effects were verified this year by observations made at the DTM installation on River Road near Seneca, Maryland, where the 22.2 and 38.7 mc/sec interferometers were stationed.

The measurements at the lowest frequency were made with an interferometer having a 50-wave-length spacing. Such a spacing is sensitive to changes in source size of less than a degree. Since the source could no longer be detected when within 25 solar radii, its apparent size must have increased from its normal 5 minutes of arc to over a degree, even at this great distance



from the sun, owing to coronal scattering.

The scattering phenomenon, being a refractive effect, must vary as the square of the wave length. Consequently, the effect, though still great, should not be so marked at the higher frequency of 38.7 mc/sec. This proved to be the case. At this frequency, two interferometers, having spacings of 27 and 90 wave lengths, were used. The greater spacing is sensitive to small changes, of the order of half a degree; the shorter spacing is useful when the source size has increased to well over a degree. By the time the apparent size of the source at 22 mc/sec had increased to

patterns were intermixed during the occultation observation, the reduction of the data required detailed study of both phase and amplitude of the trace. The reduction has not been completed as this report is written, although it is evident that the amplitude did not decrease so far from the sun as it did with the longer spacing.

As these measurements were proceeding at the River Road (Seneca) site, the solar interferometers at the Derwood Laboratory were adapted to observe the effect at 207 mc/sec. Here the problems involved are considerably greater, for not only is the effect much smaller, but the confusion

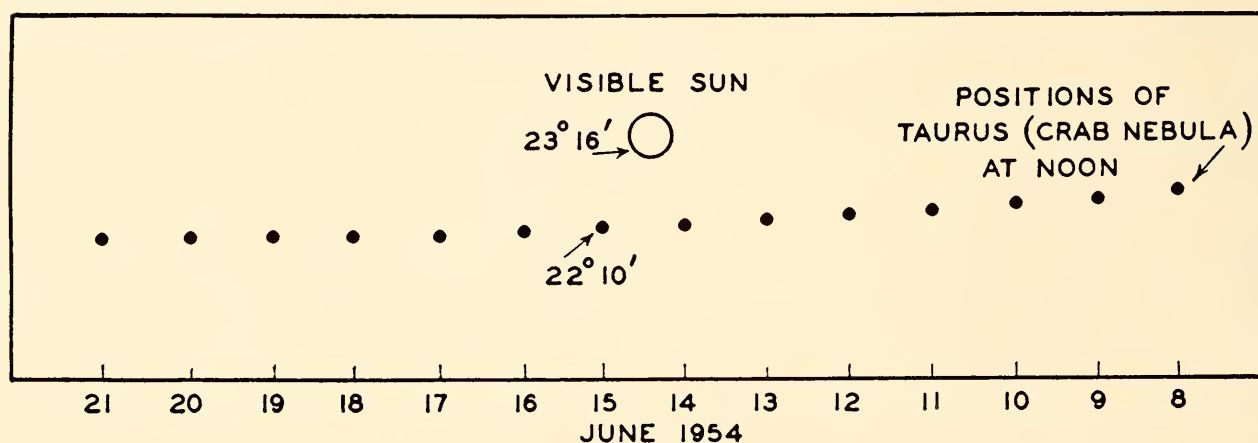


FIG. 1. Occultation of Taurus by the solar corona, June 1954

the point where it could not be seen, a small effect began to be visible on the long-spacing interferometer at 38.7 mc/sec, but the source had not completely disappeared at this spacing until it was within 5 solar radii. As expected, the source as seen on the short-spacing interferometer at 38.7 mc/sec did not increase in size so rapidly, an observation which confirms the conclusion that the effect observed in the outer corona is indeed a scattering and not an absorption phenomenon. Reduction of the data for this spacing is more difficult, however, since the quiet sun is also visible, having nearly half the magnitude of Taurus. The removal of the solar effect is made more difficult by the day-to-day variation in the sun's intensity, which may be as large as a factor of 2, even when no active regions are visible. Since the two

problem is much more serious. The intensity of the Taurus source decreases with increasing frequency, while the intensity of the sun increases, and at this frequency the sun is many times stronger. An interferometer output is proportional to the amplitude of the Fourier transform of the brightness distribution at the frequency  $d/\lambda$ ; consequently it was hoped that if the Fourier transform of the brightness distribution had a zero, the interferometer spacing could be chosen such that the solar amplitude would be zero. This was not achieved, the minimum amplitude being approximately that of the Taurus source. Thus the problem of reducing the data became more serious. Though the detailed analysis of the records is not complete, no marked effect has been identified at 207 mc/sec.



## ACTIVE AND QUIET SUN, 207 MEGACYCLES

During most of 1953 an interferometer operating at 207 mc/sec and with a spacing of 50 wave lengths was in use at the Derwood Laboratory. Radiation from the quiet and active sun was recorded for many months while the instruments and antennas were gradually modified and improved. Since February 1954 this interferometer has been in continuous operation, recording the solar transit each day. A similar interferometer with a longer base line (about 175 wave lengths, or 828 feet) also was placed in continuous operation beginning in February. This long-base-line instrument was designed to allow accurate measurements of the positions on the sun of sources of enhanced radiation.

The only period of enhanced radiation during this year occurred March 14-17, 1954, and good observations were recorded. These measurements established the important fact that the position of the localized source on the sun was not fixed, but moved about, sometimes moving 50,000 km in 3 minutes. Accuracy of measurement was better than 10 seconds of arc, and observed motions were about 1 minute of arc.

It is planned to extend the base line of the interferometer to about 300 wave lengths in order to obtain more frequent position determinations and to reduce the effect of the quiet sun in the records.

With the sun near the minimum of its 11-year activity cycle, the solar interferometer was used to measure the brightness distribution across the quiet solar disk. The amplitude of the interferometer trace is being obtained as a function of antenna spacing. From this information the radial brightness distribution can be obtained, assuming circular or elliptical symmetry. The curve of trace amplitude vs. antenna spacing has been measured by several other groups of workers, but there is considerable disagreement regarding the form of the curve in the 100 to 200 wave-length range. One result already obtained is that the interferometer trace actually reverses phase

as the antenna spacing is increased past about 100 wave lengths. This result is important in deducing the brightness distribution of the sun from interferometric measurements.

## LOW-FREQUENCY SPECTRA

The measurement of the spectra of various celestial radio sources has recently been extended to high frequencies by Hagen and Haddock at the Naval Research Laboratory. This has resulted in a clear differentiation between two types of radio source. Similar extension of the spectrum is possible toward lower frequencies, where the information we hope to obtain concerns the radiation process of the more powerful sources; there are also problems of the ionized hydrogen clouds which might be solved by suitable measurement of absorption at low frequencies. Accurate measurements of energy flux from radio "stars" are scarce at any frequency, and are almost nonexistent below 80 mc/sec. It appears feasible for us to extend our observations down to 20 mc/sec, and possibly even to 10 mc/sec, using the River Road site, 20 miles northwest of Washington.

A receiver on 22 mc/sec has proved successful, and the flux of the two most intense sources is now being roughly determined. Future plans involve a simple but accurately calibrated antenna system, and will require a careful calibration of the standard of noise power. Receivers for 15 mc/sec and 10 mc/sec are under construction and test.

PENCIL-BEAM ANTENNA FOR 20 MEGACYCLES  
("MILLS CROSS")

The best approach to a high-resolution survey at relatively low frequencies such as 20 mc/sec appears to be a crossed linear array such as that built by Mills in Australia. This receiving system employs two linear dipole arrays of great length, each of which gives a fan-shaped beam. The outputs are fed into a phase-switching

receiver, effectively multiplying the polar diagrams of the two arrays; hence, if the arrays are at right angles to each other, a narrow pencil beam is obtained. The two arrays can be oriented in many ways, the most obvious being a simple symmetrical cross oriented either north-south and east-west, or at  $45^\circ$  to these directions. The result in either case is a pencil beam, the direction of which depends on the phasing of the arrays.

Alternatively, the two arrays could be displaced to form a T or L, or even completely separated. These nonsymmetrical arrays are actually hybrids between pencil beam and interferometer, the polar response depending on the distance between the centers of the two arrays.

Although each type has its advantages, it was decided in the course of our search for a suitably analytical receiver system for low frequencies that the symmetric cross would be best, primarily because interpretation of the records is somewhat simpler. The final orientation, a slightly flattened X (see plate 1), was decided largely by the available site—a large field adjacent to the site near Seneca, Maryland, where the other low-frequency interferometers are in operation. Design of the antenna was completed and construction started in June 1954; near the end of July preliminary records were obtained (see fig. 2).

Each array has an over-all length of 2048 feet, and consists of 64 folded dipoles equally spaced, with two dipoles omitted in the center to reduce interaction between arrays. The resolving power of such an aperture should be about  $1^\circ$ . The dipole feed is different from that used by Mills, who fed all dipoles into a single low-loss transmission line, capacitively tapped to provide loose coupling to the dipoles. In the present case, each group of four dipoles is brought together and matched to 52-ohm coaxial cable; subsequently the groups are paired through equal lengths of cable, and the resultant 26 ohms transformed back to 52 ohms. The pairs are in turn paired, the process continuing, pyramid-wise, until

the two halves are brought together in a final junction. The great advantage of this system is the ease of phasing the dipoles, since the beam can be swung simply by inserting line lengths at appropriate places.

#### INSTRUMENTATION

##### *Stability Tests on 200-mc Systems*

When an interferometer is used for position measurements, errors may be introduced if the cables to the two antennas change length (for example, if temperature changes cause expansion of the cables) or if the preamplifiers or other circuit elements vary in the amount of phase shift which they produce. In order to measure the phase drifts occurring in the Derwood solar interferometer, calibration cables were installed to the antennas and correlated signals fed into the preamplifier inputs. The phase difference at the ends of the cables was then continuously measured. The results indicated that the required stability of  $\pm 5$  electrical degrees was easily obtainable for periods up to a day. Records of radio stars indicated that the antennas themselves did not contribute any appreciable phase drifts, so that measurements with an over-all accuracy of 10 seconds of arc can be made.

##### *Helical Antennas*

Eight antennas of the Kraus "helical beam" type have been constructed and installed at the Derwood Laboratory for use in the solar program at 200 mc/sec. These antennas have many advantages over other types, such as economy of shop time, very wide band, and ease of accurate duplication. These antennas were employed in the Taurus-source occultation experiment mentioned earlier. For this experiment they were arranged to produce the smallest possible solar record while still recording the passage of the Taurus source.

##### *Receivers and Components*

The receivers used for radio astronomy have been modified to incorporate many



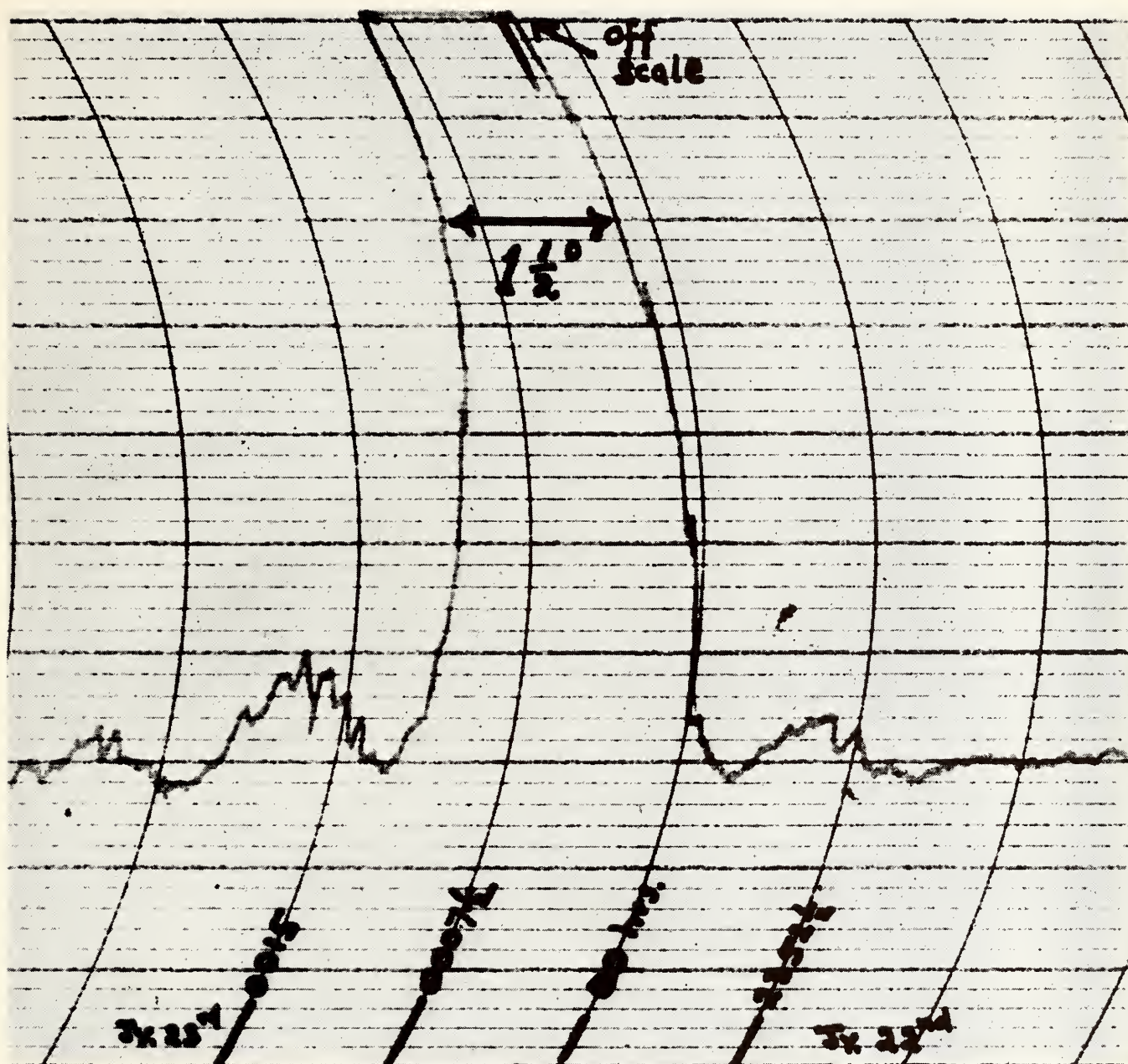


FIG. 2. Radiation from Cygnus A at 22.1 mc/sec. Recorded on the crossed-interferometer array at Seneca, Maryland, July 23, 1954.



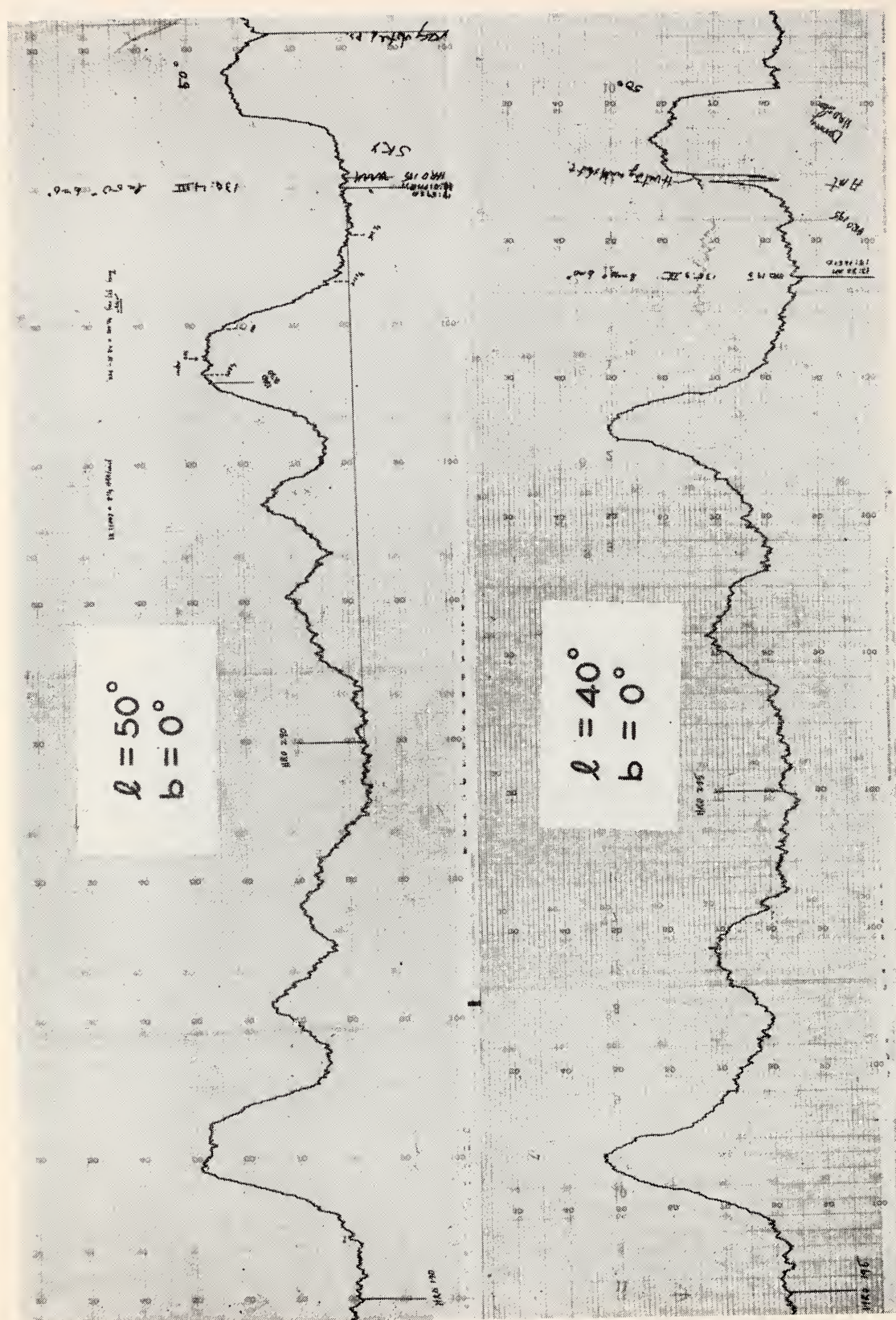


FIG. 3. Doppler-shifted hydrogen 21-cm emission lines observed from two points in the Galaxy. These curves, obtained at this Department, show antenna temperature (intensity) as the ordinate and frequency as the abscissa. A run is made by first sweeping up in frequency and then sweeping down in frequency. Consequently, the line profile and its mirror image are drawn. At the extreme right a 50° K signal, used for intensity calibration, appears. Since any velocity shifts of the hydrogen are related to the distance of the hydrogen, and the cold hydrogen gas occurs chiefly in spiral arms, each emission hump in the tracings corresponds to a separate galactic spiral arm.



desirable features of British and Australian apparatus. Also, it has been possible to achieve a great deal of simplification with improved performance. Considerable development has been completed here on preamplifiers, including the low-noise cascade RF heads, the mixers, and the intermediate-frequency amplifier. A unit packaging arrangement permits a high degree of flexibility.

Several types of electronic switch have been developed and tested under operating conditions. The Cavendish capacity switch and the Australian "Mills type" crystal switch, however, continue to perform very satisfactorily. Possible advantages of other switches being tested are improved simplicity (ease of construction) and greater sensitivity in some cases. A prototype receiver has been constructed, incorporating the unit packaging features with a 1000-cycle switching rate and suitable filters in the audio stages. The next major change for the 200-mc equipment will be a shift of the intermediate frequency to 30 or 40 mc from the 15-mc channel now being used. The change would eliminate possibility of interference from ionospherically propagated signals, which have caused occasional interruptions to solar recordings.

#### RADIO OBSERVATIONS OF HYDROGEN CLOUDS IN OUR GALAXY

H. E. TATEL, M. A. TUVE, AND H. L. HELFER

The observation of the only radio frequency line thus far found, the line produced by neutral hydrogen atoms at a wave length of 21 cm, promises to be of extreme importance in revealing information concerning the dynamics and spiral structure of our Galaxy. Observations made at the Department of Terrestrial Magnetism during the summer of 1953 confirmed the appearance of the hydrogen-line profiles found by the group at Leiden at several points, particularly at  $l=40^\circ$ ,  $140^\circ$ ,  $150^\circ$ , and  $190^\circ$  ( $b=0^\circ$ ). The tracings obtained at  $l=190^\circ$  show more pronounced minima than do the Leiden

profiles. Since both observatories use a Wurzburg paraboloid, the difference is probably attributable to the smaller band width Carnegie uses (15 kc/sec rather than 40 kc/sec). The tracings at  $l=40^\circ$ ,  $b=0^\circ$  compared with off-galactic plane tracings at the same longitude indicate that the hydrogen in the distant circular arm, about 10 kiloparsecs from the sun in this direction, according to the Leiden observers, extends at least 1.0 kiloparsec in a direction perpendicular to the plane, and that the plane of symmetry is about a degree above the galactic plane.

Operations ceased in September 1953 for necessary repairs, and in December construction was begun on a new receiver, which was put into operation in the early part of the summer of 1954. The design of the receiver, described above under "Instrumentation," is such as to insure much greater stability. The use of a modulated RTMA 6144 noise diode permits placing a standard  $50^\circ$  K deflection on the recorder. The preliminary runs indicate an antenna temperature of  $70^\circ$  K rather than  $100^\circ$ , as previously reported in the literature, for the main peak of the standard point at  $l=50^\circ$ ,  $b=0^\circ$  (see fig. 3). Observations in the region  $l=100-120^\circ$ ,  $b\cong 0^\circ$  show clearly that the spiral arm nearest to us appears to be split into two branches. Preliminary observations in the direction of the north galactic pole failed to show any hydrogen emission in the normal frequency range, implying that the sun is probably in a region of low hydrogen density and that the hydrogen intensity in the spiral arm situated about 500 parsecs away from the sun falls off very rapidly in our direction.

#### THE UPPER ATMOSPHERE

B. F. BURKE, J. W. FIROR, L. OWREN,  
F. G. SMITH, AND H. W. WELLS

##### SCINTILLATIONS

Radio-star scintillation of the radio waves from discrete celestial sources has been observed as consistently as possible

during the year, using simple techniques at 38 mc/sec. The scintillation results from irregularities in the upper ionosphere, and to understand the origin of the irregularities one must investigate their behavior over different parts of the earth and their incidence at different times. Results for the latter part of 1953 were compared with local ionospheric data, and with results for similar experiments in Cambridge, England. For these places the over-all character of the phenomenon is the same, and a close correlation with "spread F echoes" was found. No hour-by-hour or day-by-day correspondences were found, however, and the diurnal observations differed considerably unless a mean curve over a period of as much as 3 months was drawn, when the curves became nearly identical.

The origin of the disturbances remains unknown.

#### IONOSPHERIC "WINDS"

The study of irregularities and movements—sometimes called winds—in the ionosphere has been continued intermittently. As usual, principal attention has been given to traveling disturbances in the F region. Several important new facts have been added to our knowledge of these strange occurrences. The operation of a fourth or "joker" station has demonstrated unequivocally the reality of the events as moving disturbances. It will be recalled that measurements of direction and velocity of moving ionospheric surfaces are made from a triangulation of stations. Data from three stations are required to obtain a value of direction and velocity of movement (assuming a plane-wave front).

The fourth station was located in the direction of prevailing movement. Arrival times were calculated from the tripartite analyses, and the apparent errors (the differences between observed and predicted values) were determined. These apparent errors, which vary from a few per cent to about 50 per cent, may be evidence of

changes in velocity or direction, of curved front or irregular shape of blob, of limitations in timing accuracy, or of combinations of these factors.

Analyses of multifrequency observations at the three stations approximately 15 miles apart show that a disturbance at any one station may be traced in depth through the thickness of the F region, and that a finite time interval exists between stations. For this spacing of stations the normal time interval varies up to 4 or 5 minutes. Knowledge of thickness of the ionospheric layer and the apparent transit time in depth permits an estimate of the vertical component of velocity. For several cases analyzed, the apparent vertical component was approximately 100 m/sec. Several independent interpretations are possible, such as a disturbance entering the ionosphere obliquely, an inclined front moving horizontally, or an inclined front moving downward.

Horizontal velocities range from 50 to 250 m/sec, and the predominant direction of movement is to the east or southeast.

Speed and direction of ionospheric winds may also be determined from the observation of radio-star scintillations at three stations located a short distance (1 to 3 miles) apart. The correlation and time displacement between scintillation records may be used for determining velocity and direction of the traveling "clouds." Similarly, an estimate of the size of the irregularities is obtained from the loss of correlation found when the distance between stations is expanded. Good progress has been made toward completion of the two additional 38-mc receivers and location of sites needed for the experiment. Tests of the system are scheduled for the fall of 1954.

#### SPREAD F ANALYSES (HUANCAYO, PERU)

As early as 1938, the occurrence of a phenomenon now known as "spread F" was described by Booker and Wells for Huancayo, Peru. The event appears to be a form of ionospheric scattering of radio



waves in the F region and is characterized by a transition from normal, clearly defined regions to rough, irregular boundaries without any clearly defined penetration frequencies. Subsequent analysis reveals pronounced diurnal, seasonal, and annual characteristics. It is fundamentally a nighttime event, with greatest frequency of occurrence in the period from 4 hours before midnight to 4 hours after midnight. The scattering is most prevalent during seasons when the sun is overhead, and is infrequently observed during May, June, July, and August (local winter), when the noon solar zenith angle becomes as great as  $35^\circ$ . The relative total annual occurrence of F-region scatter for the period 1938 through 1945 shows low values during 1941-1942, followed by a rapid increase through 1946, which is not closely related to solar activity. The diurnal properties of F scatter correspond closely to reported characteristics of radio-star scintillations, with peak activity around midnight. The annual or seasonal properties, however, are not in simple agreement. Properties of spread F are also being investigated by Grote Reber in Hawaii.

## THE EARTH'S CRUST

### SEISMIC STUDIES

H. E. TATEL, M. A. TUVE, AND P. J. HART

The course of our seismic exploration of the earth's crust has changed many times in the past eight years. It has changed from the textbook stage of identifying each large wiggle in a seismogram as a real structure, through the antithetical position of doubting whether the whole seismogram contained any usable information, to our present position. This is that a small but interesting amount of real information about the earth can be obtained by seismic observations if they are obtained in considerable numbers and have a suitable geographic spread. The first position led to the textbook picture of an orderly layering of the crust, but this became contradictory and absurd when many observations were

made. The opposite position led to a concept of a completely disorganized crust. In our reports for the past two or three years a position somewhere between has been reached, and a sounder physical basis for our conclusions can now be expounded.

The major difficulty experienced in the interpretation of the seismograms arises from the complexity of the recorded ground motion after an explosion (or an earthquake). From the quasi-coherent patterns observed in the multiple seismograms, we had hypothesized that much of this ground unrest was caused by the conversion of upcoming compressional (P) waves converting into slower surface or Rayleigh (R) waves at the earth's uneven and broken surface. The slower R waves tended to persist as they passed the observing point, giving rise to the extreme and extended ground motion, particularly between the epoch of P wave and that of R wave arrivals. A test of this hypothesis is to measure the ground motion. One could then determine whether or not the conversion-produced R waves do appear just after the P wave and before the regular R wave.

As outlined in last year's report, we had constructed two portable strain seismometers to be used in conjunction with a vertical seismometer. The principle of operation as first worked out by Benioff would enable us, in certain simple cases, to determine the nature of the ground motion. The use of the strain seismometers turned out to be difficult. Some rock, apparently part of large massive structures, gave no strain indication. Since a rock must show strain if a wave passes along it, there was no true wave passage. Yet the inertial seismometers read properly, and we concluded that large rock masses of this type must be "floating" upon the earth's true surface. These apparently solid masses, which cannot be larger than hundreds of feet, must therefore go up and down like corks on the water as the seismic waves pass.

In three localities we did obtain good

strain seismograms at 100 km from the explosion. Though the records tended to be complex, there was good evidence of Rayleigh wave motion just after the arrival of the P wave. The only manner in which such slow waves could arrive so quickly was through some conversion process, the energy traveling speedily as P waves for a large part of the way. Thus encouraged, we decided at this point to try a model experiment in order to test these hypotheses of conversion under controlled conditions.

The model earth we use is a block of steel or brass with the smallest dimension (4 to 8 inches) sufficiently large so that all waves of interest are recorded before the arrival at the recording point of reflections from any surface of the block. Transmitters and receivers ("seismometers") are made of specially cut and polarized barium titanate. The piezoelectric property of this material is used to form a mechanical impulse by the sudden application of an electric voltage. Conversely, the mechanical strain impressed on the receiving barium titanate crystal by the passing wave gives rise to an electric voltage in the receiver, which is amplified and applied to a high-speed cathode-ray oscilloscope (0.1 microsecond resolution or better). Since electrical impulses are used to form the mechanical pulses, the oscilloscope is simultaneously triggered, and time-exposure photographs can then be taken of repeated (500–2000) cathode-ray sweeps.

The transmitter and receiver in our model experiments on Rayleigh wave conversion were typically 5 to 15 cm apart. The impulse duration was 0.2 to 0.5 microsecond, or a Heaviside pulse of comparable rise time. The effective size of the transmitter and receiver was 0.2 cm. The velocity of the compressional waves turned out to be 0.6 cm per microsecond, or 6 km/sec. The duration of the received pulse was about 1 microsecond and the pulse train was thus 0.6 cm, hence the experimental conditions were satisfactory; the transmitter and receiver were of di-

mension less than a wave length, and their separation was much greater than a wave length.

The first result of the model experiments was a verification of Lamb's theory. In 1903 Horace Lamb published an analysis of the ground motion at distances remote from a surface impact (fig. 4). His analysis showed a very simple pattern as compared with his observations, and he remarked that the theoretical curves "show nothing corresponding to the long successions of to-and-fro vibrations" characteristic of the records of seismographs.

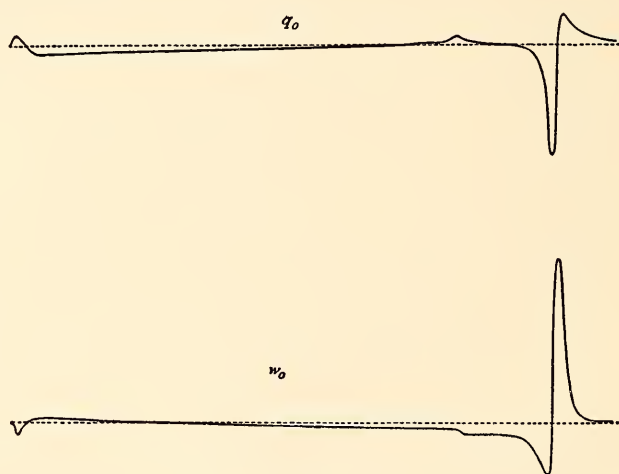


FIG. 4. Lamb's (1903) ground motion from a distant impulse. Upper curve, horizontal motion; lower curve, vertical motion.

In the seismograms obtained here (fig. 5) on the iron-block model, the motion was quite similar to that predicted by Lamb. Impulses corresponding to the compressional (P) and Rayleigh (R) waves were found, and these maintained their correct relation during various shifts of position; indications of the shear (S) waves were also found. In addition, a peculiar motion or "slow recovery wave" between P and S was observed for the first time. This was shown not to be a wave of velocity intermediate between P and S, as the mathematics seemed to indicate, but a surface wave—probably a Rayleigh wave.

In one model we drilled into the surface a series of small holes, of depth less than a wave length, between transmitter and receiver. A subsequent seismogram then



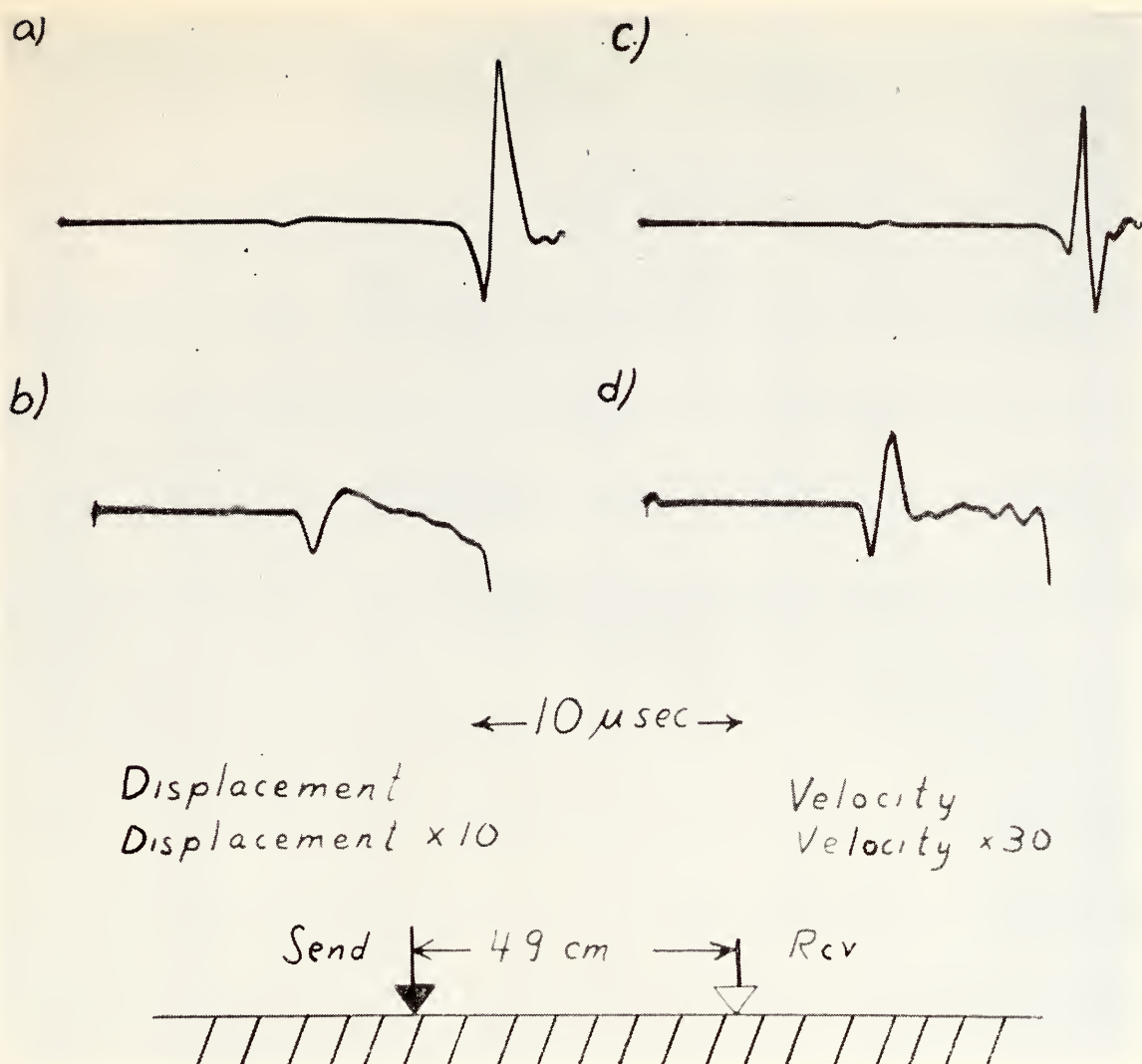


FIG. 5. Model seismograms showing the vertical component of surface motion from a point impact, as in Lamb's theory; source size much less than the wave length and reception distance much greater than the wave length. Seismogram (a) is to be compared with Lamb's prediction " $w_0$ " (fig. 4).

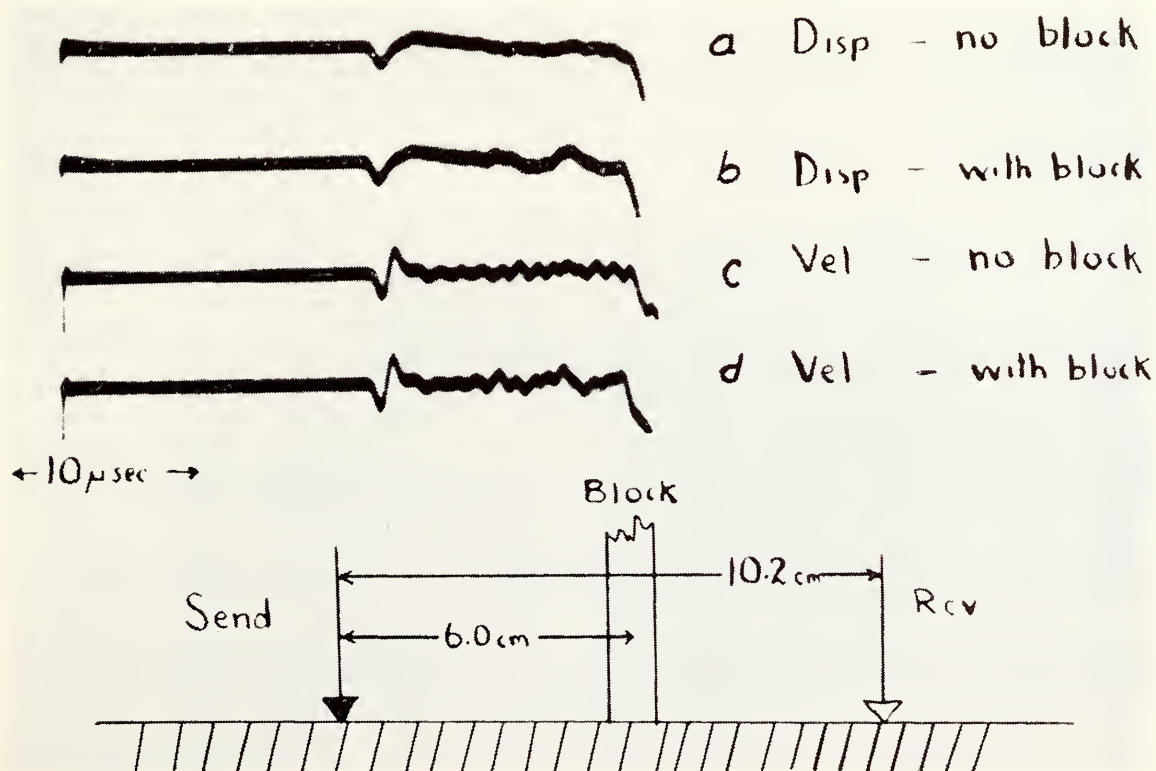


FIG. 6. Seismograms showing the effect of a surface disturbance by a surface obstacle of wave-length dimensions; the compressional wave produces a Rayleigh wave, and the Rayleigh wave produces a compressional wave at the obstacle.





had an appearance very much like that of a field seismogram, differing greatly from the ideal or simple one the same block had given before the holes were drilled. Thus, surface "topography" or near-surface obstructions were shown to have marked effect on a seismogram, as our field experience had led us to believe.

In order to determine the mechanism of the effect, single scattering centers were used (fig. 6). These were in the form of long, rectangular metal bars. The small face,  $0.5 \times 1.5$  cm, of a bar with the smaller dimension along the line from transmitter to receiver was stuck to the iron block (the model earth) with a soft grease. Each "mountain" of this kind gave rise to two pulses. Measurement of time and distance indicated that these were the results of two conversion processes at the surface of the model earth: a conversion of a P wave to an R wave, and of an R to a P wave. The first conversion, P to R, we had concluded from our field work was a strong effect. The model showed that the R to P conversion was similarly important. These conversion pulses are comparable with the P wave itself, though smaller in amplitude than the primary R wave from the source. A series of several "mountains" makes a series of these conversion pulses and diminishes the size of the primary R wave to that of the P wave. The resultant seismogram is then a series of "to-and-fro vibrations." The P wave is not greatly affected; it is a body wave continuously carrying energy from the interior to the surface. Conversely, the primary Rayleigh wave, being imprisoned in the surface, has no large energy supply.

Thus many aspects of a seismogram can now be explained. Near a shot there are pronounced P and R waves. As the waves progress they convert at the surface and form other waves which cause more ground motion during the time interval between the arrival of the primary P and the primary R waves. This surface-induced ground motion is a hodgepodge of R and P motion. As the primary Ray-

leigh wave progresses outward from the source, it is always losing energy into secondary P waves which radiate into the interior of the earth, and the primary P wave coming to the surface converts to R waves, the conversion being strongly dependent on the surface topography. Thus the amplitude of primary R waves is diminished in distance with respect to the P wave. Observations of shots in deep wells have shown the absence of Rayleigh waves—as was well known—and the diminution of reverberation after the normal Rayleigh wave. Thus the reverberation occurring after the R wave from a surface shot may be attributed in large part to R-wave scattering. This picture accounts rather well for all our experimental observations to date, including about two thousand seismograms recorded some tens and hundreds of kilometers from various shot points.

It is clear that this cannot be the whole explanation, for some scattering is to be expected from structures within the earth. In order to estimate the relative contributions of internal scattering and of surface conversion waves to the "reverberation" seen on every typical seismogram, we have made recordings on the surface from an oil-well shot at 2000 feet depth, and also recordings at 3000 feet depth in several open wells at distances of 1 to 3 km from a gas-well shot at a depth of 3000 feet in northwestern Pennsylvania. These observations have enabled us to eliminate to some extent the effect of the surface, as these depths are considerably greater than a Rayleigh wave length for our system (1000 feet). The deep shot and deep receiver combination shows a limited reverberation, which at a distance of 1 to 2 km from the shot ceases abruptly at 1 second after the P arrival. This result is encouraging, for it is the decrement of the reverberation with respect to time which we wish to determine. The decrement observed for this explosion was clearly greater than for the ordinary case of surface explosion-surface seismometer. The

amplitude of the explosion was insufficient, however, to show long-continued reverberation above the ground noise level, so we have not yet been able to obtain a good measure of the reverberation decrement of deep shot-deep seismometer as compared with that of the deep shot-surface seismometer observing arrangement. It is this latter comparison, for which the Rayleigh waves are greatly diminished in both cases, which may enable us to estimate the relative contribution to reverberation which arises from interior scattering.

The extremely low noise level 3000 feet down a well, namely 5 per cent of the surface value, or even less, offers a possibility for observation of vertical reflections. These experiments are not easily arranged, as an explosion shot of 1000 pounds or more at well depth would seem to be required, and it is problematical whether or not such shots can be carried out. The drop in noise amplitude with depth is a good indication that most of the surface ground unrest in the frequency spectrum of 6 to 30 cycles per second is Rayleigh wave motion. We are indebted to Mr. Frank Eckert and Mr. John Woods and their associates of Hanley and Bird, Bradford, Pennsylvania, and also to Mr. J. W. Bird and his associates of Bird Well Surveys, Bradford, Pennsylvania, for permission to use their gas wells and shots, and for their very helpful participation in the observations.

This year we undertook to obtain many careful measurements on either side of the critical distance for reflections from the base of the crust near Washington. The critical distance is that at which the angle of reflection from the base of the crust becomes critical and the reflection abruptly approaches total reflection. Previously we had endeavored primarily to establish the existence of the total reflection in various directions from different shot positions. When we came to analyze the data in our records, we found that what we had in hand was not sufficiently definitive to establish the nature of the discontinuity.

More data, however, have not led to the critical distinction we sought, namely, that between an abrupt discontinuity and a less abrupt transition region.

We find that the data may be well fitted by two models. In one model the velocity, starting at the surface at 6 km/sec for the compressional wave, increases slowly to about 7 km/sec at a depth of 30 km. At this depth there is an abrupt increase to 8 km/sec at the outer mantle. In the other model, the velocity of 6 km/sec at the surface remains constant to a depth of some 24 km. The velocity at this depth then begins to increase, with most of the velocity change occurring in a zone of about 3 km depth just above the outer mantle boundary, at 30 km depth, where the velocity of 8 km/sec is attained. The first model is the usual one of a crust with a sharp transition giving the phenomenon of critical reflection. The second model has a zone at a depth of 24 to 30 km where the rays are refracted and focused to give the strong second arrivals which we observe and call "reflections." Because of the inhomogeneities within the earth, the data are not precise enough to permit a choice between these models on the basis of the travel-time curves alone.

The critical distance has been found to be close to 80 km in the Chesapeake Bay area. Previously we had found it to be approximately 88 km. A few reflections are found at less than 80 km, in fact as close as 70 km. These, however, are of smaller amplitude and somewhat sporadic. If these are true critical reflections, then the velocity need not increase at all with depth in the crust; the simple discontinuity would explain all the data. It is, however, our judgment that these reflections at less than 80 km are not total. They are either partial reflections, in the case of the first model, or some sort of scattering or "scintillation" of ray paths in the second model.

There are of course many other more complex hypotheses which may be introduced to explain the data. These can involve stepwise zones of increasing or de-



creasing velocity at different depths down to 40 km. But if such layers exist, they are not actually discontinuous, for their boundary reflections definitely are not observed; we have made an extensive search for such effects and have found no systematic evidence for layering. Thus these more complex hypotheses or models come in pairs. First the model with its more complex hypothesis is introduced, then a complementary idea is advanced to explain the failure to observe some of the most characteristic properties of the proposed model, such as reflections. Of course newer analyses or data from other sources may ultimately allow us to choose from many of the possible models.

#### ISOTOPE DATING OF ANCIENT IGNEOUS INTRUSIVES

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AND G. W. WETHERILL

The co-operative program of staff members of the Department of Terrestrial Magnetism and the Geophysical Laboratory which aims to ascertain age relations between igneous intrusives of the Pre-Cambrian era has completed its third year. It can be safely stated that the initial goals of the program are within the reach of the group. We have now measured the age of one such intrusive near Ohio City, Colorado, by all the radioactive decay schemes thought to be practicable. Thus we know the calculated uranium-lead and lead-lead ages of a zircon from this region, the rubidium-strontium age of several lepidolites, and the potassium-argon age of a microcline. This is the type of information we have been accumulating in these early stages of the work, in order to assess the relative reliability and ease of measurement of ages so determined. In addition to the age-measurement work, the interests of the group have gradually broadened to include the study of the geochemistry of these radioactively related elements. From these

studies we hope to learn something of the time differentiation of such elements in the crust, the degree of mixing of products of weathering, and the relative amounts of two such related elements in the crust. Since isotopic abundance measurements are the key in these studies, the same physical and chemical tools may be applied to these questions as to those of age relationships. In fact, the two types of studies are so intimately woven together that proper interpretation of the measurements involved in determining age relationships requires knowledge of the geochemistry of the two elements, parent and daughter. This report will be divided according to the parent element concerned in each phase of the work, since the work is in part so divided among the staff members.

#### *Potassium-Argon Mineral Ages*

One of the three naturally occurring isotopes of potassium,  $K^{40}$ , is radioactive and disintegrates into either  $Ca^{40}$  or  $A^{40}$ , the first mode of disintegration being about 10 times as probable as the latter. Since potassium is an abundant element in the crust of the earth, the possibility of radioactive dating using potassium minerals is very attractive. Techniques have been developed here during the past year for accurately measuring the potassium and argon content of potassium minerals. The knowledge of these potassium and argon concentrations, together with a knowledge of the decay constants and the assumption that the potassium and argon have been retained since the formation of the mineral, permits the age of the mineral to be calculated. The possibility of dating some of these same minerals by measuring the quantity of calcium which has been formed by the decay of potassium is under consideration. One gram of a potassium mineral such as microcline will contain about 100 mg potassium, and, if it is 1000 million years old, will contain about  $5 \times 10^{-4}$  cc at standard temperature and pressure of radiogenic argon. This quantity of radiogenic argon is measured by an

<sup>1</sup> Geophysical Laboratory, Carnegie Institution of Washington.

isotopic dilution technique similar to that used for rubidium, strontium, uranium, thorium, and lead in other parts of our program. Potassium measurements can be made either by isotopic dilution or with a flame photometer.

The argon is released from the mineral by vacuum fusion of the mineral in a nickel furnace, using sodium hydroxide as a flux. The gases which are evolved from the melt are equilibrated with a known amount of  $A^{38}$  tracer. The tracer is obtained from the Atomic Energy Commission, which produces it by neutron bombardment of chlorine in a nuclear reactor. Once the tracer is equilibrated with the argon released from the mineral, yield losses will not affect the argon determination. The mixture of gases is then purified of water, oxygen, hydrogen, carbon monoxide, and hydrocarbons, by means of a purification train consisting of magnesium perchlorate and cupric oxide traps, and a tungsten filament operated at  $2000^{\circ}\text{C}$ . The residual gas mixture contains only the inert gases and nitrogen. The gas is then adsorbed on activated charcoal contained in a break-off tube, and sealed off. The sample is then sealed onto the sample-control system of a mass spectrometer, and the argon isotope ratios are measured. From the ratio of the  $A^{40}$  peak to the  $A^{38}$  peak (introduced as a tracer) the quantity of  $A^{40}$  can be determined. The presence of normal nonradiogenic argon is indicated by the  $A^{36}$  peak; usually the argon is about 98 per cent radiogenic. All gas handling is done with a greaseless glass vacuum system using no stopcocks or ground-glass seals, in order to avoid the possibility of leaks or occluded gas in the stopcocks or in the grease.

The  $A^{38}$  tracers are prepared by filling twenty 10-mm O.D. break-off tubes with a mixture of  $A^{38}$  and Xe, the  $\text{Xe}/A^{38}$  ratio being about 100. The function of the xenon is to saturate the adsorptive power of the glass walls and prevent adsorption of  $A^{38}$ . These tracers are filled on a common manifold and therefore are all at the

same pressure. The volume of each individual break-off tube is accurately measured, and therefore the ratio of the quantity of gas in the various tubes is accurately known. The absolute calibration of any one of the tubes will then suffice to calibrate them all. This absolute calibration is made by mixing one of the  $A^{38}$  tracers with a sample of spectroscopically pure atmospheric argon of known pressure and volume, measured in a specially constructed McCleod gauge. The analysis of this gas mixture in the mass spectrometer gives the information necessary for calculating the quantity of  $A^{38}$  in this one tracer, and hence in all the tracers. This calibration is repeated several times to check the reproducibility of the method.

A difficulty that arises in potassium-argon age determinations is the uncertainty in our knowledge of the decay constants of potassium,  $\lambda_{\beta^-}$ , the probability per unit time of the  $K^{40}$  atom's decaying into a  $Ca^{40}$  atom, and  $\lambda_K$ , the probability per unit time of the  $K^{40}$  atom's decaying into an  $A^{40}$  atom. These decay constants can be determined by counting experiments, the accuracy of which we hope will improve in the future. It is also possible, however, to obtain information concerning these decay constants from the experiments with minerals. Samples of microcline, biotite, and uraninite have been collected from the same pegmatites. The uraninites can be expected to give unambiguous uranium-lead ages, and the decay constants of  $K^{40}$  can be chosen to make the potassium-argon age of the biotite and microcline fit the uranium-lead age of the uraninite. Another method is to measure the ratio of radiogenic  $A^{40}$  to radiogenic  $Ca^{40}$  in a suitable mineral; this measurement gives the branching ratio,  $\lambda_K/\lambda_{\beta^-}$ , directly, with no dependence on the age of the mineral.

Both of these methods of determining the decay constants, as well as the success of the potassium-argon age method itself, depend on the validity of the assumption that the greatest part of the radiogenic



argon is retained within the mineral in which it is formed. This can be checked by comparing the values of the decay constants calculated from measurements on different mineral specimens. It is unlikely that samples of different types of minerals, of different age and of different history, would leak argon to the same extent. Thus, reproducibility of the calculated decay constants would be strong evidence against argon leakage. The number of measurements made here and elsewhere are not sufficient to settle this question at this time. They do, however, offer hope that the assumption of argon retention is valid. In view of the great potentialities of the argon-age method, it is necessary to obtain the answer to this question as soon as possible.

Actual measurements are nearing the end of the preliminary stage, and the results of the two "age" determinations which have been made are shown in table 1 for two possible branching ratios,  $R$ .

To facilitate these measurements, a new mass spectrometer, to be used exclusively for gas analysis, is being constructed. Several special features are being incorporated in this instrument, such as differential pumping of the source and analyzer vacuum systems, an electron multiplier detector, and higher (5000 v) accelerating voltage. It is expected that this instrument will be in regular use in the autumn of 1954.

### *Rubidium-Strontium Mineral Ages*

The work on the rubidium-strontium decay scheme for age determinations may be divided into three parts. The first is a simple extension of the number of locations for which ages have been measured by the rubidium-strontium decay procedure. Three new granite locations have been dated from a determination of the  $Rb^{87}$  and  $Sr^{87}$  in biotite. The results are given in table 5 at the end of this section, since uranium-lead ages have also been determined for these same granites and the comparisons are more important than

the ages from any one decay scheme. From the biotite work we have confirmed our ability to determine  $Sr^{87}$  at a level of one part per million. We have also found that the radiogenic  $Sr^{87}$  content of the strontium in biotite can be expected to be much larger than had previously been found elsewhere. We attribute this result to the great care taken in the mineral separation procedures at the Geophysical Laboratory.

Further work on lepidolites this year has included duplicate analyses of the same mineral, demonstrating that the reproducibility of the analytical results is well

TABLE 1  
POTASSIUM-ARGON AGES

| MINERAL    | LOCATION                  | AGE (MILLION YEARS) |           |
|------------|---------------------------|---------------------|-----------|
|            |                           | $R=0.085$           | $R=0.125$ |
| Lepidolite | Bikita, Southern Rhodesia | 3140                | 2500      |
| Microcline | Brown Derby Mine, Colo.   | 1080                | 780       |

within the 5 per cent limit which is accepted as satisfactory and desirable for this work. Table 2 shows such data for two minerals of different rubidium content from the Harding Mine at Dixon, New Mexico. As has been previously reported,

TABLE 2  
REPLICATE DATA ON MINERALS FROM HARDING MINE, DIXON, NEW MEXICO

| Date            | Wt. of sample (mg) | Rb (mg/g) | $Sr^{87}$ ( $\mu g/g$ ) | Age (million years) |
|-----------------|--------------------|-----------|-------------------------|---------------------|
| LEPIDOLITE      |                    |           |                         |                     |
| June 25, 1953.. | 205.0              | ...       | 41.0                    | ....                |
| April 6, 1954.. | 94.0               | 7.67      | 40.2                    | 1660                |
| April 13, 1954. | 100.7              | 7.93      | 40.1                    | 1610                |
| MUSCOVITE       |                    |           |                         |                     |
| May 28, 1953..  | 103.7              | 6.29      | 31.5                    | 1590                |
| April 19, 1954. | 76.9               | 6.35      | 32.0                    | 1610                |



the ages of intrusives as measured by the rubidium-strontium decay scheme have often been greater than those found from the uranium-lead decay. In an effort to determine whether the reason for this apparent discrepancy could be found, five occurrences of lepidolite, of different color and grain size, from the Brown Derby Mine at Ohio City, Colorado have been analyzed for rubidium and strontium. The analytical results are shown in table 3. The only marked discrepancy in age is in the fine-grained lilac material. Its  $\text{Sr}^{87}$  content is exactly that of the coarse lepidolite embedded in a matrix of quartz and feld-

TABLE 3

RUBIDIUM-STRONTIUM DATA FOR LEPIDOLITES FROM  
BROWN DERBY MINE, OHIO CITY, COLORADO

| Specification                  | Rb<br>(mg/g) | $\text{Sr}^{87}$<br>( $\mu\text{g/g}$ ) | Age<br>(million<br>years) |
|--------------------------------|--------------|---|---------------------------|
| Fine grain (lilac) . . . . .   | 21.4         | 133                                     | 1980                      |
| Fine grain (white) . . . . .   | 21.8         | 118                                     | 1730                      |
| Medium grain . . . . .         | 21.2         | 113                                     | 1700                      |
| Coarse (in feldspar) . . . . . | 24.1         | 134                                     | 1760                      |
| Coarse books . . . . .         | 19.5         | 110                                     | 1790                      |

spars. These two occurrences are from the same hand specimen and would be expected to be of exactly the same age. The rubidium content of the coarse lepidolite is over 10 per cent greater than that of the fine. It is further seen that the age of this fine-grained material is 10 per cent greater than that of all four other occurrences. This is at least an indication of some rubidium leakage from the finest-grained material. The other ages are so nearly the same that no significant differences due to grain size can be said to occur.

It is seen in tables 2 and 3 that the ages for the Harding Mine and the Brown Derby Mine are less by almost exactly a factor of 2 than those reported last year for these same two locations. An error in one of the calibrations affecting these particular results of last year was found after that report was made. The confusion between the two reported values is regret-

table. The calibration error arose from confusion as to the amount of strontium spike solution which was added in a few of the analyses. The age of the Bonneville, Wyoming lepidolite is still measured to be 3000 million years, giving quantitative evidence of truly ancient Pre-Cambrian rocks in the United States.

The third phase of the rubidium-strontium analytical program is the continuation of the investigation of the strontium isotope abundances in rocks of different ages begun two years ago by Herzog at our laboratory. He looked at the rubidium-free feldspars in the granites which he was studying for radiogenic  $\text{Sr}^{87}$  in the rubidium-containing minerals, and found that the  $\text{Sr}^{87}$  content of these feldspars was much too high for the supposed age of the granite which contained them. The work this year has been on sedimentary rocks of both recent and ancient origin.

The chemical separation of the trace amounts (0.02 to 0.1 per cent) of strontium from the bulk of calcium was achieved by means of appropriate resin ion-exchange columns. The mass spectrometric analyses were performed exactly as in our earlier work. Samples of early Pre-Cambrian South African marble, middle Pre-Cambrian Grenville marble, Ordovician and Devonian limestones, and modern sea water have been analyzed. The change in the abundance of the  $\text{Sr}^{87}$  isotope is less than one-half of that expected from (a) the best assumption as to the relative abundances of rubidium and strontium in the crust, (b) the decay constant of  $\text{Rb}^{87}$ , and (c) the assumption that the average isotopic composition of strontium in the ocean is the same as that of strontium in the crust at any given time. Figure 7 shows mass spectra of strontium obtained from sea water and from the South African Pre-Cambrian marble. The difference in the mass 87 peaks results from change in the abundance of this isotope due to additions of  $\text{Sr}^{87}$  from the decay of  $\text{Rb}^{87}$  over the 2.5 to 3.0 billion years since the South African marble's deposition. The

predicted difference in the  $\text{Sr}^{87}$  content between these two samples was 10 to 12 per cent instead of the 2 or 3 per cent apparent from the mass spectra.

The fact that the results do not agree with the values predicted by the assumptions indicates either that one or more of the latter is not valid or that they are insufficient. Additional measurements should

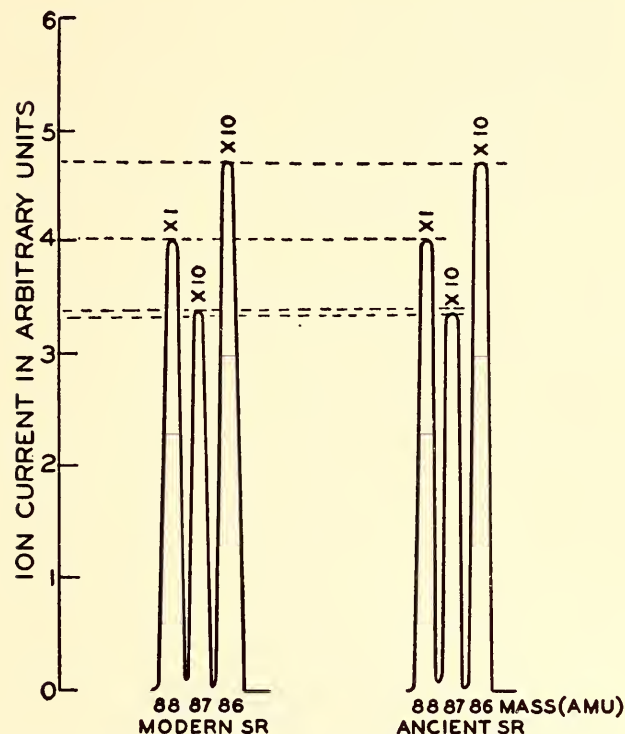


FIG. 7. Mass spectra of strontium from modern sea water and an ancient South African marble. The small difference indicated in the mass 87 peaks of the two samples is less than one-fourth that predicted a few years ago before any such measurements as these had been made. This difference results from additions of  $\text{Sr}^{87}$  to strontium in sea water over the time elapsed since the South African marble was laid down.

provide the clue to the difficulty and shed new light on the geochemistry of both rubidium and strontium.

Future work on the rubidium-strontium decay scheme will be largely centered in the comparative age studies until its dependability has been established.

#### *Uranium-Lead Mineral Ages*

Techniques and facilities for the determination of lead on the microgram scale by the method of isotope dilution

were completed in the past year. Ion-exchange techniques which have previously been used for the separation of rubidium and strontium from minerals for age measurements have been extended to enable the isolation of lead as well. The results of the new lead-age work appear in table 5 at the end of this section.

The zircons were chosen for study because of their close field relation to micas, which permits independent age determinations by the rubidium-strontium method. It was found that the zircons gave low uranium-lead ages relative to their own lead-lead ages. The fact that all these zircons are of a fine grain size (200-350 mesh) is believed to be significant. In previous work, zircons several millimeters in length from another granite gave uranium-lead and lead-lead ages which agreed within 5 per cent. More work with coarser zircons is planned in the coming year to test whether grain size is a critical factor in the age discrepancies. In particular, if it is possible to obtain fine and coarse zircons from a single specimen or to obtain fine zircons from an area where the age is already well established, it should be possible to establish whether the uranium-lead or the lead-lead ages are the more accurate. We shall then be in a position to draw conclusions concerning the causes of these discrepancies. A field trip to the Grenville subprovince of the Canadian Shield in June 1954 has provided a number of zircon-bearing specimens which may meet the requirements outlined above.

The two monazites reported in table 5 were obtained from pegmatites in the same region of South Africa as the lepidolites from Kinderzitt and Jakkalswater reported a year ago. The lepidolites gave derived rubidium-strontium ages of 1210 and 1100 million years, respectively. The monazites appear to give somewhat younger derived ages. Since the monazites and lepidolites were obtained from separate pegmatites, it is possible that they are not contemporaneous. It is interesting that



the agreement of the thorium-lead and uranium-lead ages is much better for these pegmatitic monazites than it was for several minerals separated from granites which were reported here a year ago. The thorium-lead ages previously reported were less than the uranium-lead ages by factors of 2 to 3.

### *Geochemistry of Uranium and Lead*

Lead ores in nature are found to contain four isotopes having masses of 204, 206, 207, and 208. It is known that the decay of thorium leads to the stable isotope  $Pb^{208}$ , whereas the decay of uranium leads to the

alter its isotopic composition since the earth was formed.

Such a lead sample may have been found in an iron meteorite. The lead data were obtained by C. Patterson of the California Institute of Technology, and the uranium analysis is from the work of Tilton at the University of Chicago. The amount of uranium present in this meteorite is too small to change the isotopic composition of the lead now present, even over a period of billions of years. This lead has the lowest ratios of  $Pb^{208}$ ,  $Pb^{207}$ , and  $Pb^{206}$  to  $Pb^{204}$  yet observed and is thus the "oldest" lead known. It is calculated, by a method in-

TABLE 4  
SOME LEAD ISOTOPIC COMPOSITIONS AND URANIUM-LEAD RATIOS

| Source  | Uranium<br>(ppm) | Lead<br>(ppm) | Lead<br>uranium | $\frac{Pb^{206}}{Pb^{204}}$ | $\frac{Pb^{207}}{Pb^{204}}$ | $\frac{Pb^{208}}{Pb^{204}}$ |
|---|------------------|---------------|-----------------|-----------------------------|-----------------------------|-----------------------------|
| Earth's crust .....                                       | 1-4              | 5-20          | 5               | 18.6                        | 15.6                        | 38.4                        |
| Troilite phase of Canyon Diablo iron meteorite .....      | 0.009            | 18            | 2000            | 9.41                        | 10.27                       | 29.16                       |
| Diopside from Dreiser Weiher, Germany, olivine bomb ..... | 0.0067           | 2             | 300             | 19.5                        | 15.5                        | 38.6                        |

stable isotopes  $Pb^{207}$  and  $Pb^{206}$ .  $Pb^{204}$  is not produced by any known long-lived radioactivity. Other laboratories have studied the isotopic composition of lead ores of different ages and have shown that young ores have considerably higher ratios of  $Pb^{208}$ ,  $Pb^{207}$ , and  $Pb^{206}$  to  $Pb^{204}$  than do older ores. Furthermore, if these ratios are plotted against the ages of the various ores, the variation of the ratios follows very closely that which would be calculated from the relative abundances of the total uranium, thorium, and lead in the crust of the earth today (see table 4). It thus appears that lead ores can be considered, to a first approximation at least, to represent a bulk sample of the total lead in a given region of the earth's crust at the time the particular ores were formed. This suggests that we should be able to measure the age of the earth if we somehow were able to obtain a sample of lead which had never been in contact with enough uranium to

dependent of the uranium-to-lead ratio of the crust, that it would require 4.5 billion years to produce lead of the isotopic composition found in the earth's crust today if it had initially the isotopic composition of the lead contained in this meteorite. One disadvantage of this approach is, of course, that meteorites are not of terrestrial origin. It would be much more satisfying to find a terrestrial lead with an isotopic composition similar to that of the iron meteorite.

With this purpose in mind, a study was made of the uranium and lead of an olivine bomb from Dreiser Weiher, Germany, supplied by Professor H. H. Hess of the Department of Geology at Princeton University. The bomb contained four minerals, olivine, spinel, bronzite, and diopside, encased in a shell of basalt. Various geologists believe that these bombs may represent samples of material from the earth's mantle, i.e. from below the Mo-



horovičić discontinuity. It was inferred from previous data in the literature that such samples should have high lead-to-uranium ratios resembling that of the Canyon Diablo meteorite. The lead-to-uranium ratio of the bomb actually confirmed expectations, but in spite of this the lead in the bomb was found to have an isotopic composition similar to that of the earth's crust at the present time. This means that the lead in the bomb has spent some part of its past history in an environment with a much lower lead-to-uranium ratio than that found in the bomb today. It is possible that the minerals in the bomb are the result of a crystal differentiation of the basalt in which it occurs, since basalts resemble the earth's crust in having lead-to-uranium ratios of 4. The basalt accompanying the bomb was too badly weathered to permit study of its lead.

A second olivine bomb from San Bernardino, California was studied in a similar manner. The lead data for this bomb were less accurate than those for the German bomb, but agreed with it within the coarser limits of error. The lead from both bombs shows significantly higher  $Pb^{206}/Pb^{204}$  ratios than would be expected from the  $Pb^{208}/Pb^{204}$  and the  $Pb^{207}/Pb^{204}$  ratios. This interesting observation is unexplained at present.

The common lead found on the earth today represents a mixture of two types: primary lead that was present when the earth was formed, and lead that has been produced by the radioactive decay of uranium and of thorium. It has been known for many years that lead extracted from minerals of different ages reflects these diverse origins by the variability of its isotopic composition. Hence, knowledge of the isotopic composition of lead present in rocks should provide a basis for establishing their time of origin. This method of dating, to be successful, would depend on the fulfillment of four essential conditions: that the lead being studied was captured by the host at the time of its origin; that it has not been augmented by further radio-

active contributions; that it has been protected from subsequent modification by solutions containing younger lead; and, finally, that the lead that was captured was a valid sample, representative of the earth's common lead at the time the rock was formed. If these considerations prove valid, the lead found in feldspars of igneous rocks should be of interest for dating purposes.

Efforts have been made to develop procedures by which the lead of feldspars, usually present at a concentration of only a few parts per million, can be easily extracted in quantities sufficient for mass spectrometric measurement (about 25  $\mu$ g). In earlier work we had demonstrated the power of ion-exchange techniques for separating rubidium and strontium from minerals for age measurements; these techniques have been elaborated so that it is now possible to extract the lead rapidly and easily from dissolved samples.

Before measurements on lead from feldspars can be carried out, however, further investigations will be needed on the problem of the absorption of lead by the platinum dish in which the sample is dissolved. Experience to date indicates that a significant fraction of these relatively minute amounts of lead may be lost to the platinum and cannot be recovered easily. Apart from the problem of yield, this behavior could upset the results owing to contamination from previous lead samples processed in the same platinum dish, or to a nonrepresentative absorption of the sample lead, or of the special "spike" lead that must be added for quantitative measurements by isotopic dilution. These problems are under examination as the report year ends, but it seems clear that reliable lead isotope determinations will shortly be feasible on the feldspars.

#### *Comparisons of Mineral Ages*

Table 5 is the cumulative result of all the measurements by isotope dilution from which comparisons of mineral ages may

be made. The data for the Namaqualand samples are given because, although the lepidolites and the monazites are not from the same pegmatite, it is believed on geological bases that the pegmatites listed are all congeneric. It is seen first that the rubidium-strontium ages are nearly always 10 to 20 per cent higher than the highest

of the uranium-lead ages, and secondly that the disagreement among the uranium-lead ages is often as large as that between the lead-lead and rubidium-strontium ages. The disagreements shown demonstrate the complexity of the problem of reliable age determinations of minerals and rock structures.

TABLE 5  
MINERAL AGE COMPARISONS

| LOCATION                        | MINERAL    | CALCULATED AGES (MILLION YEARS)     |                                     |                                      |          |                                      |
|---------------------------------|------------|-------------------------------------|-------------------------------------|--------------------------------------|----------|--------------------------------------|
|                                 |            | U <sup>238</sup> /Pb <sup>206</sup> | U <sup>235</sup> /Pb <sup>207</sup> | Pb <sup>207</sup> /Pb <sup>206</sup> | Rb/Sr    | Th <sup>232</sup> /Pb <sup>208</sup> |
| Cape Town, South Africa         | Zircon     | 350                                 | 375                                 | 550 ± 50                             |          | ...                                  |
|                                 | Biotite    | ....                                | ....                                | .....                                | 820 ± 40 | ...                                  |
| Uncompaghre, Colorado           | Apatite    | 1050                                | 1380                                | 1810 ± 160                           |          | ...                                  |
|                                 | Biotite    | ....                                | ....                                | 1650                                 | 1650     | ...                                  |
| Bagdad, Arizona                 | Zircon     | 630                                 | 750                                 | 1190 ± 70                            |          | ...                                  |
|                                 | Muscovite  | ....                                | ....                                | .....                                | 1640     | ...                                  |
|                                 | Lepidolite | ....                                | ....                                | .....                                | 1900†    | ...                                  |
| Ohio City, Colorado             | Zircon     | 935                                 | 1110                                | 1480 ± 110                           |          | ...                                  |
|                                 | Lepidolite | ....                                | ....                                | .....                                | 1750     | ...                                  |
| Steenkampskraal, Namaqualand    | Monazite   | 1090                                | *                                   | *                                    | ....     | 990                                  |
| Goodhouse District, Namaqualand | Monazite   | 930                                 | 920                                 | 880                                  | ....     | 900                                  |
| Kinderzitt, Namaqualand         | Lepidolite | ....                                | ....                                | .....                                | 1210     | ...                                  |
| Jakkalswater, Namaqualand       | Lepidolite | ....                                | ....                                | .....                                | 1100     | ...                                  |

\* The quantity of radiogenic Pb<sup>207</sup> was too small relatively to the primary Pb<sup>207</sup> in the sample to permit an accurate determination of this age.  
† The result given is revised from the value reported last year on the basis of new Rb determinations.

THEORETICAL AND STATISTICAL GEOPHYSICS

SITES OF CURRENT SYSTEMS RESPONSIBLE FOR  
GEOMAGNETIC DISTURBANCE  
E. H. VESTINE

Two new results were derived which may have a crucial bearing on theories of magnetic storms. These are the finding by V. C. A. Ferraro and M. Sugiura that the sudden commencement of storms is abnormally augmented during sunlit hours at the geomagnetic equator, as shown by data from Huancayo, Peru, and the finding by Vestine that the same augmentation phenomenon appears during the initial hours of a magnetic storm, or initial phase. In fact, both these effects closely resemble or even exceed the augmentation at the equator of the ordinary quiet-day diurnal variation, which is today attributed to the

electric currents in the E region of the conductive ionosphere. These electric currents are presumably generated by the dynamo action of ionospheric winds, moving electrically conducting air across the geomagnetic field. Hence the same may be true in the sudden commencement and initial phase of storms. At any rate, the highly localized magnetic field pattern at the ground during sudden commencements and the initial phase of storms irrefutably indicates (from potential theory) that the major immediate source of field on the ground must be electric currents flowing in the ionosphere, and not chiefly electric currents at a distance of several earth radii, as is sometimes supposed.  
Of course eastward-flowing electric currents may appear both in solar streams and



in the atmosphere. One possibility is that the atmospheric currents close at hand, yielding most of the magnetic field changes at the ground, may in some way be induced by changing magnetic fields of external origin; this will make necessary complex additions to existing solar-stream theories, and it will be interesting to see whether or not these become somewhat forced as a result. Hence new evidence supports the finding in last year's report that about 75 per cent of the field changes of magnetic storms observed at the ground must be ascribed to electric currents in the atmosphere, with the possible exception of the mainly equatorial part of storms averaged around parallels of latitude.

#### GENERAL CIRCULATION OF THE ATMOSPHERE

H. L. HELFER AND E. H. VESTINE

It is known that the rotation of the solid earth fluctuates with time. In particular, it undergoes a seasonal fluctuation in rotation rate. This is ascribed to seasonal changes in the angular momentum of the troposphere, referred to the earth's axes of rotation. If then this tropospheric motion is coupled to and can impress itself upon the solid earth, it may also communicate its motion to the ionosphere, in accordance with the principle of conservation of angular momentum. It is hence of interest to attempt estimates of the effects of the kinematic viscosity and electromagnetic couples on the relative zonal motion of air at various heights, and it is also desirable to calculate the motion transmitted from the troposphere in this way, in some typical case. Unfortunately, this problem, like most problems related to the general circulation of the atmosphere, is very complex, so that results can be given only for a highly idealized case.

One special case studied is that in which the solid earth, supposed cylindrical, undergoes a periodic fluctuation in rotation, assumed to be communicated to an exponential atmosphere. It was found that even in this case the disturbance is propa-

gated upward in a complicated way, the zonal motion changing from westward to eastward alternately as the height increases, until near maximum amplitude actual slippage of the atmosphere as a whole may be considered to take place. This is expected to occur when the wind velocity changes by an amount equal to itself within a vertical range equal to the average distance between molecular collisions. This curious result is being compared with the reversals in zonal wind velocity with height determined by Whipple from the motions of meteor trains. It is also hoped to extend these results to dynamo theories of the geomagnetic fluctuations and to hydromagnetic phenomena. In another study, begun by Vestine while at the Cavendish Laboratory, slippage phenomena in a region of vertical temperature contrast are shown to give rise to wave motions as in billow clouds (or turbulent rollers), nearly independent of the zonal motion of the neutral gas, but dependent on vertical density contrast. It is hoped that these wave motions can explain the density contrasts in electric-charge concentration producing radio-star scintillations near the 400-km level of the ionosphere. This work is also being extended to include the calculation of the electric and toroidal magnetic fields occasioned by the supposed slippage layer.

#### DYNAMO THEORY OF THE GEOMAGNETIC VARIATIONS

E. H. VESTINE

A preliminary attempt was made to construct a revised dynamo theory of magnetic disturbance. Using Kelvin's circulation theorem, it was shown that transient equatorial heating of the upper atmosphere may give rise to rapid poleward meridional air flow. If the return circuit is also meridional at a higher level in a conducting region, the initial phase of magnetic storms may arise there from the eastward-flowing electric currents generated by dynamo action. Within an hour or so, under continued accelerating action, the meridional



circulation should become mainly zonal, owing to the Coriolis force, and with the motion mainly from east to west. The electric field will then be directed equatorward, and should give rise to westward-flowing Hall currents increasing in strength until the maximum of the main phase of the magnetic storm is reached. The counteracceleration of the zonal winds will then be expected to cause the wind system to decay with time, more rapidly at first, during the recovery period of a magnetic storm. This recovery in the geomagnetic field observed during geomagnetic storms should have nearly the same time constant as the supposed wind system, since the time constant of electric current systems freely decaying in the atmosphere would be much shorter than the period of one or two days observed.

#### COSMIC-RAY INVESTIGATIONS

S. E. FORBUSH

*Cosmic-ray intensity variation with sunspot cycle.* An analysis of the long series of reduced cosmic-ray data from Godhavn (1939-1949), Cheltenham (1937-1952), Huancayo (1937-1952), and Christchurch (1937-1952) indicates for the first time a world-wide variation of cosmic-ray intensity with range nearly 4 per cent, negatively correlated with sunspot numbers. This variation was found to be similar at all four stations. The existence of this effect was heretofore obscured by linear drifts, due probably to the decay of long-life radioactive contaminants in the instruments; these drifts were different at the several stations. Evidence also was found to indicate that there may be a sunspot-cycle variation in the northward component of the earth's magnetic field, in phase with that found for cosmic-ray intensity. Vestine had previously found that there is evidence indicating such a variation in the earth's field, but that its determination is uncertain on account of the unpredictable vagaries of the secular variation. Nevertheless, the correspondence sug-

gests that the mechanism responsible for the sunspot variation in cosmic-ray intensity and that in the earth's field are closely related; the maximum and minimum of cosmic-ray intensity occurred respectively in 1944 and 1947, which were years of sunspot minimum and maximum. For Huancayo, which provides the most reliable daily mean values, the curve of daily means for 1947 is, in general, everywhere well below that for 1944. This indicates that the decrease in intensity in 1947 is not due to magnetic-storm effects on cosmic-ray intensity. In the latter event it would be expected that the intensity in 1947 would, between storms, more often recover to values as high as those in 1944.

*World-wide cosmic-ray intensity variations.* High correlations were found between the monthly mean values of cosmic-ray intensity at any two of the four stations. The standard deviation (variability) of daily means from monthly means at Huancayo was found to be at a minimum, about 0.2 per cent, at sunspot minimum in 1944, and about three times as great near sunspot maximum. In 1944, for example, the variability of daily means at Christchurch and Cheltenham was obviously much greater than at Huancayo, particularly in the winter months, and at Godhavn the variability of daily means was even greater than at Cheltenham or Christchurch. The greater variability at the latter stations probably arises from greater variations in the vertical distribution of air mass (the systematic variation of which gives rise to the seasonal waves in cosmic-ray intensity). This variation may increase (or decrease) the height (around 16 km) of the 100-millibar layer, at which most  $\mu$ -mesons are formed, and consequently may increase (or decrease) the number which decay (half life 2 microseconds) before reaching the apparatus, thereby increasing (or decreasing) the apparent cosmic-ray intensity. Though these effects may obscure small variations, such as a 27-day recurrence variation of 1 per cent amplitude which can be reliably seen at Huancayo,

they seldom obscure the larger changes of 2 per cent or more which are often associated with magnetic storms. Nevertheless, when averaged over several intervals of time the variations at Godhavn, Cheltenham, and Christchurch are well correlated with those at Huancayo. Thus any real variations in daily means of cosmic-ray intensity (except for increases observed during a few solar flares, seasonal waves, and  $\mu$ -meson effects) which are *not* world-wide must be very small, probably not larger than a few tenths of one per cent.

*Search for geomagnetic effects predicted from a model by Alfvén to explain cosmic-ray variations in magnetic storms.* In a model proposed by H. Alfvén, the decrease in cosmic-ray intensity during magnetic storms, in essence, was explained as follows: a conducting stream of gas ejected from the sun carries with it ("frozen in") a magnetic field (from the neighborhood of a sunspot, for example); the stream moves with a velocity of about  $10^8$  cm/sec, corresponding to a travel time of about 1 day from the sun to the earth. An observer (or a cosmic-ray particle) not moving with the stream would, while such a stream passes by, experience an electric field. The magnitude of this electric field depends on the strength and direction of magnetic field in the stream, the streaming velocity, and the width of the stream. Thus on crossing the stream the energy of a cosmic-ray particle would be changed by a calculable amount. Special investigation of the orbits of those cosmic-ray particles which can reach the earth after penetrating through its magnetic field leads to the conclusion that the energy of the cosmic-ray particles would suffer only a decrease. It was suggested in discussions with Alfvén that the electric field responsible for the consequent decrease of intensity might also affect the flow of those electric currents in the auroral zone which give rise to the well known disturbance diurnal variations ( $S_D$ ) in the earth's magnetic field.

A few storms were studied according to whether or not they were accompanied by

decreases of cosmic-ray intensity. For each hour of the storm, the magnetic horizontal vector departure (with quiet-day diurnal variation removed) from the quiet-day average as origin was plotted from Godhavn magnetic data. The magnitude of this horizontal vector varies little throughout the day for  $S_D$  averaged for seasons or years, and its phase progresses uniformly during the day. In the individual storms of the two groups investigated, the magnitude of this vector varied greatly during the day (as might be expected during magnetic bays, for example). Its phase progressed, however, in a very uniform fashion throughout the day and in a way which was quite similar whether or not the storm was accompanied by a decrease of cosmic-ray intensity.

Since this study was completed, Swann has shown, for a simple model of the solar stream, that if the width, velocity, and magnetic field strength in the solar stream were such as to alter appreciably the energy of a cosmic-ray proton crossing it, then the proton could not cross the stream because its trajectory would be coiled up on account of the magnitude and extent of the magnetic field in the stream.

Attempts to determine whether the southward extent of the auroral currents is different in storms with and without decreases in cosmic-ray intensity also failed.

*Statistical study of reliability of Compton-Bennett data and comparison with neutron-counter data.* The fact that the variability of daily means of cosmic-ray intensity at Huancayo is less than at Cheltenham, Christchurch, and Godhavn has already been mentioned together with the reasons for it. For the year of minimum sunspot numbers, 1944, the standard deviation of daily means from monthly means at Huancayo was about 0.2 per cent, and less than for any other year in the period 1937-1952. This figure places an upper limit to the combined effects, on cosmic-ray intensity at Huancayo, of any variability of vertical air-mass distribution there, uncertainties in scaling records, and the un-



avoidable sampling errors arising from the statistical nature of the arrival of cosmic-ray particles at the instruments. Using some unpublished data from a Compton-Bennett meter at Teoloyucan, Mexico, daily means for ten months of the year 1937 were corrected for seasonal variation and drift. The standard deviation of single differences of daily means (for the same day) from their average monthly difference was found to be 0.24 per cent. If the variance (squared standard deviation) of the difference of daily means is divided equally between Huancayo and Teoloyucan, the standard deviation of daily means (after deducting world-wide changes) is only about 0.17 per cent; this indicates a relatively small uncertainty in daily means at Huancayo and at Teoloyucan.

Some neutron-counter data obtained by Simpson at Sacramento Peak, New Mexico provided daily means for June 1951, during which month there was a range of about 5 per cent in neutron intensity. There was a high correlation between the daily means of neutrons at Sacramento Peak and the daily means of ionization at Huancayo, although the range in ionization at Huancayo was 0.39 times that in the neutron counts. The standard deviation of the difference between the daily means of neutron counts and ionization was found to be about 0.25 per cent. Using the figure 0.17 per cent for the standard deviation of a daily mean at Huancayo, a standard deviation of 0.47 per cent is derived for the daily mean of neutron counts. Since the neutron percentage changes at Sacramento Peak were 2.5 times as great as the percentage changes in ionization at Huancayo, the ratio of the changes to their uncertainty was for the month of June 1951 about the same at both places. Thus for indicating world-wide changes in daily means, the Compton-Bennett data at Huancayo appear about as reliable (on the basis of this small sample) as those from neutron-counter data.

Neher has published data from several balloon flights obtained over a period of

several years at Omaha, Nebraska and Saskatoon, Canada. These showed changes of about 20 per cent under  $140 \text{ g/cm}^2$  of air. When these data were compared with changes in daily means at Huancayo for the same dates, the correlation was found to be excellent, with the changes on the balloon flights about 10 times those at Huancayo.

*Old cosmic-ray program.* Compton-Bennett cosmic-ray meters were satisfactorily maintained co-operatively in continuous operation throughout the report year at Godhavn (Greenland), Cheltenham (Maryland), Huancayo (Peru), and Christchurch (New Zealand). Reduction of bi-hourly means of cosmic-ray intensity has been completed through 1953. Tabulations of bi-hourly means for Huancayo from 1946 to 1953, and for Cheltenham from 1937 to 1952, are ready for publication, as well as summaries for Godhavn and Christchurch. Publication of these data will provide a valuable addition to similar results through 1946 contained in CIW Publication 175, and will thus make available to investigators most of the essential data obtained since the start of the Department's cosmic-ray program. The cosmic-ray meter originally installed at Climax, Colorado in 1949 was set in operation in September 1953 at the High Altitude Observatory, Climax, after being out of operation about two years. One additional Compton-Bennett meter is being made ready for installation at the University of Mexico, in Mexico City. The University has provided a building for housing the meter and will undertake proper maintenance.

*Large ionization chamber.* The large cosmic-ray ionization chamber was maintained with continuous registration at Derwood during the report year. No large or small "flares" were observed.

*Neutron meters.* One neutron meter was kept continuously operating at Derwood during the report year. No sudden increases during solar flares were found. A second meter with a lighter and im-

proved lead-paraffin pile and with improved arrangements for keeping the insulators dry was tested, and will be set up for continuous operation at Derwood. Though these meters should be more effective than the Compton-Bennett meters for detecting increases that may occur during solar flares, yet, because of the requirement for constant and high insulation, they will very likely prove inferior to the Compton-Bennett meters for revealing world-wide changes in intensity over long periods of time. Control boxes were completed for use with additional neutron meters for use at other locations as detectors of increases during solar flares.

*Co-operation in operation of cosmic-ray*

*meters.* We acknowledge the fine co-operation of the following in operating cosmic-ray meters: Godhavn (Greenland), the Danish Meteorological Institute and the staff of its Godhavn Magnetic Observatory; Cheltenham (Maryland), the U. S. Coast and Geodetic Survey and the staff of its Cheltenham Magnetic Observatory; Huancayo (Peru), the Government of Peru and the staff of its Instituto Geofísico de Huancayo; Christchurch (New Zealand), the Department of Scientific and Industrial Research and the staff of its Christchurch Magnetic Observatory; and Climax (Colorado), the staff of the High Altitude Observatory of Harvard University and University of Colorado.

## LABORATORY PHYSICS

### NUCLEAR PHYSICS

N. P. HEYDENBURG AND G. M. TEMMER

#### COULOMB EXCITATION OF NUCLEI

During the past year we have studied energy levels in many nuclei, ranging in atomic number up to 92. This represents a departure from the more conventional investigations of light nuclei (atomic number less than 10), which are usually considered the proper domain of nuclear physics in the three-million-volt region.

Our invasion of this domain, which was usually considered to be that of nuclear spectroscopy, i.e. of the study of beta and gamma radiation emitted from radioactive nuclei, was the result of the discovery by Huus and Zupančič (1953) that low-energy charged particles are able to excite low-lying excited levels of target nuclei through the relatively long-range inverse-square (Coulomb) electrostatic interaction. Once excited, these nuclei emit gamma radiation, usually back to the ground state, or by a cascade of gamma rays through some intermediate state; the delay will depend on the lifetime of the level in question, but for our purposes can be considered infinitely short. Our facilities are ideally suited to studies of this special kind

of nuclear excitation. The energies and intensities of the gamma radiation emitted after Coulomb excitation can be measured to an accuracy of a few per cent, using the sodium iodide scintillation crystal, whose light output is linear with energy expended within it. When this output is then sorted by some type of pulse-height selector, we have a gamma-ray spectrometer with a resolution of 8 to 10 per cent and an intrinsic efficiency which, depending on the energy, may be 30 per cent or higher (see experimental set-up in fig. 8).

Using these procedures, we have examined some 80 different nuclei in the past seven months, that is, we have obtained the pulse-height distributions and hence the energy distributions of gamma rays emitted under low-energy alpha-particle bombardment. The main interest here is in the following results: (1) number, location, and relative intensities of the various gamma rays emitted; (2) excitation curve, as a function of bombarding energy, of some of these gamma rays, either to confirm the theory or to learn about the type of transition represented by the gamma ray; (3) absolute cross-section determination, equivalent to a lifetime measurement for the inverse transition, to



determine nuclear transition probabilities. We shall here discuss various aspects of these determinations.

*Theory of Coulomb excitation.* To date, the only theory available for the so-called electric or Coulomb excitation of nuclei is a semiclassical one, based on the assumption of "geometrical optics" for the incident particles. More quantitatively, this demands that

$$\frac{Z_1 Z_2 e^2}{\hbar v} \gg 1, \quad (1)$$

where  $Z_1$ ,  $Z_2$  are the charges of the projectile and target, respectively, and  $v$  is

hyperbolic orbits exist and that the energy ( $\Delta E$ ) given up to the nucleus is small as compared with the energy carried by the passing particle. The important parameter in these calculations turns out to be the ratio of the collision time to the nuclear period, which is represented by the following combination of quantities:

$$\xi = \frac{Z_1 Z_2 e^2}{\hbar v} \cdot \frac{\Delta E}{2E}. \quad (2)$$

It should be emphasized that the cross section for Coulomb excitation consists of two parts, only one of which is governed by what we have just described, namely the details of the excitation mechanism; the other and more interesting part is the nuclear transition probability  $B_e(2)$  (square of the matrix element), which tells us about the relative ease with which a nucleus undergoes a certain transition. Now it is our objective to obtain the values of these transition probabilities  $B_e(2)$ ; in order to do this, however, we first make certain that the Coulomb excitation part of the cross section is properly understood. If this can be established, then the listing of the various matrix elements of the nuclei studied represents the completion of at least a major phase of this type of experiment. So far, no use whatever has been made of any specific nuclear model. Different nuclear models will provide definite predictions for the quantities  $B_e(2)$ , and it is there that theory and experiment can best meet. A word should be added concerning previously available methods for finding  $B_e(2)$ . There is essentially but one other, namely lifetime determination, most readily applicable to longer-lived isomers, but brought into the range of  $10^{-9}$  seconds by fast electronic coincidence techniques. In most of these measurements one is at the mercy of the particular beta-ray decay preceding the event of interest, and, furthermore, the really large transition probabilities (short lifetimes) are at present outside the range of measurement. Large transition probabilities of course mean large cross sections for Coulomb

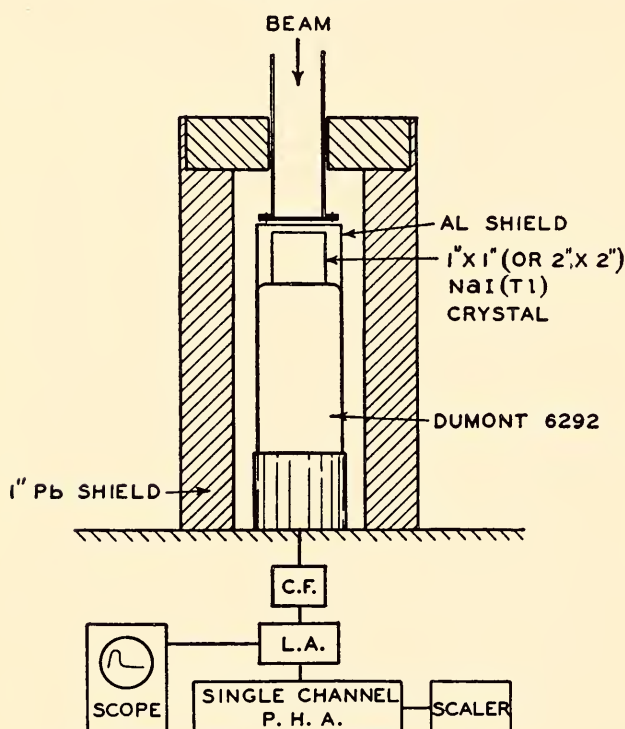


FIG. 8. Experimental arrangement for scintillation counter detection of gamma rays.

their relative velocity. This theory also assumes, and we have confirmed the assumption, that the excitation process takes place almost exclusively through electric quadrupole matrix elements, i.e. it connects only levels of the same parity whose spins vectorially differ by two units. It turns out that this selection rule is not very selective and allows us to reach most low-lying levels of nuclei.

The classical theory is, more specifically, based on the assumption that definite

excitation, so that the latter process is ideally suited for a study of these fast transitions. It is precisely these fast transitions which enter prominently into the so-called collective model of the nucleus (A. Bohr and B. R. Mottelson), about which we shall have more to say below.

*Experimental confirmation of the theory.* We have examined a number of nuclei and compared the experimentally measured cross section vs. energy curves with the simple theory. These cross sections can easily vary by a factor of over 1000 in the bombarding energy range from 1 to 3 Mev; nevertheless we have found rather good agreement over the entire range of energies, with some departures which are undoubtedly ascribable to the breaking down, for lighter nuclei, of some of the assumptions made in the theory. On the whole, however, the agreement is such as to give us confidence, both in the essential adequacy of the theory to yield transition probabilities, and in the confirmation of the electric quadrupole (E2) nature of the transitions we observe, one notable exception being the first excited state of fluorine.

Most of this work was done with targets which are thicker than the range of the incident particles, but still thin as compared with the emerging gamma radiation. In order to have a check on the correctness of our procedure for reducing thick-target results to equivalent thin-target cross sections, we have undertaken a study of thin-target excitation functions in the lighter elements (fluorine, sodium, titanium, manganese, and germanium), making use of a number of enriched isotopes. Again we find satisfactory agreement with the theory for the lower bombarding energies ( $E < 2.5$  Mev), with characteristic departures, on the high side of the theory, at the higher energies; as an example, see the curve for  $\text{Mn}^{55}$  in figure 9. These departures, as we have pointed out, are not unexpected, and can be understood on the basis of at least two processes: (a) some compound nucleus formation over and above the Cou-

lomb excitation can add to the yield of the gamma radiation observed (inelastic scattering); (b) because of the wave nature of the incident particles, there is some penetration into the Coulomb barrier, raising the excitation; some recent theoretical work, however, shows this effect to be rather unimportant. These departures are of interest in their own right, but do not contribute to our knowledge of nuclear

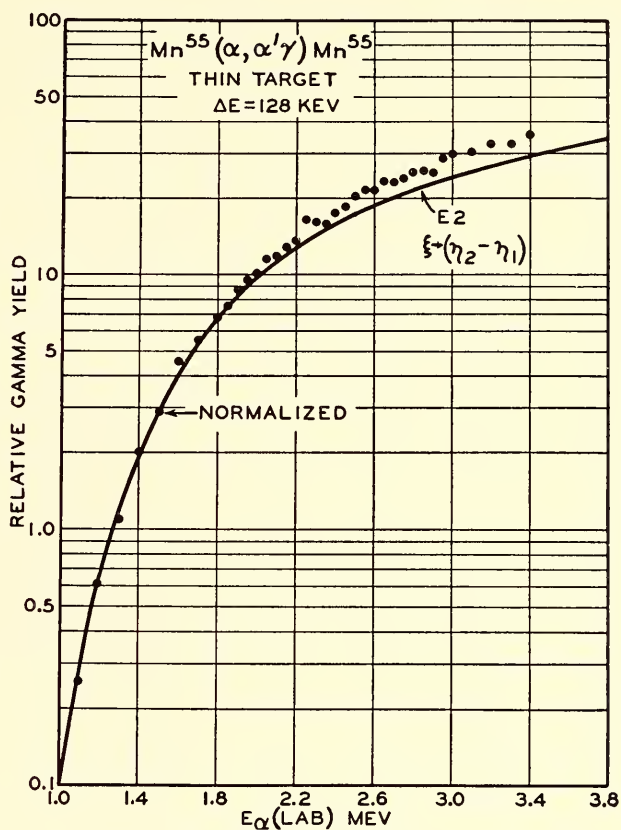


FIG. 9. Coulomb excitation by alpha particles of the 128-keV gamma ray from a thin target of manganese.

matrix elements. In the cases of fluorine and sodium we were able to compare a concurrent compound-nuclear reaction with the Coulomb excitation of energy levels, using the  $(\alpha, p\gamma)$  reaction, as will be set forth below.

*Protons vs. alpha particles as projectiles.* It is worth while to discuss briefly the reasons for the great superiority of alpha particles over protons in Coulomb excitation, which has made it possible for us to study almost all nuclei on the periodic table, whereas workers using protons have had to confine themselves to the heaviest ele-



ments (atomic number above 60): (a) Because of their higher charge, and lower velocity at a given energy, alpha particles satisfy condition (1) down to much lower atomic number, and hence make it possible to use the simple theory for most nuclei. (b) The strong characteristic X-radiation due to K-shell ionization which is always present when we bombard our targets (see sample pulse-height distribution in figure 10) is only about one-tenth as strong as with protons. This radiation interferes with the detection of very low energy

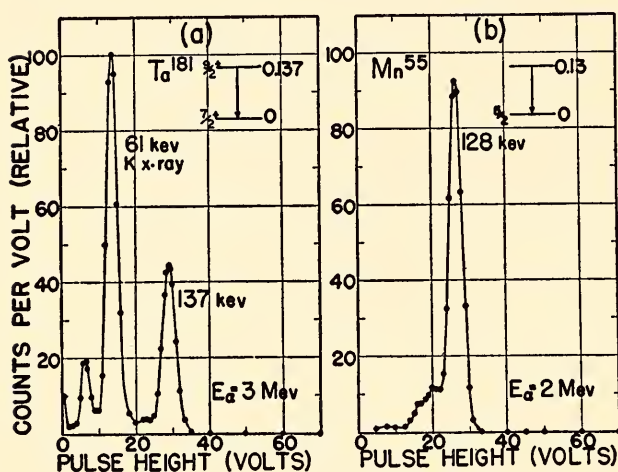


FIG. 10. (a) Pulse-height distribution of 137-keV gamma radiation and 61-keV K X-radiation from Ta<sup>181</sup> bombarded by 3.0 Mev alphas. (b) Pulse-height distribution of 128-keV gamma radiation from Mn<sup>55</sup> bombarded by 2.0 Mev alphas.

levels, and makes it impossible even to locate certain levels in the heavy elements. (c) Many of the medium-heavy elements have thresholds for the (*p,n*) reaction between 1 and 2 Mev, interfering with gamma-ray detection; of course, (*p,nγ*) processes may also occur which leave the residual nuclei excited, and gamma radiation then follows which belongs to a different nucleus and confuses the situation. (d) Protons produce capture radiation of the order of 7 Mev, up to atomic number of about 40, making detection of low-energy radiation very difficult. (e) The Coulomb barrier for alpha particles is twice as high as for protons, and hence there is a much larger region of excitations available in which the Coulomb excitation process is

essentially the only one producing transitions to excited states. (f) The problem of finding inert backing materials for the support of thin targets is minimized. (g) Finally, the general background radiation from the accelerator is much lower when alpha particles are used, mostly for the reasons given under (d) and (e). As far as actual thick-target yield at a given energy is concerned, protons are considerably more effective in exciting higher-lying levels (their penetration into the target is greater) and can be used to advantage in the heavier nuclei.

We shall now discuss what experimental verification we have been able to find for one of the consequences of the collective model of the nucleus as proposed by Bohr and Mottelson. Without going into any detail, we may say that this model attempts to take into account not only the irrefutable aspects of the nuclear shell model, but the liquid-drop properties of nuclei as well, which are evident in the fission process. When we are far away from closed shells—and the region of the rare earths with mass numbers lying between 150 and 200 is one of these—we are to expect a low-lying level structure, associated with a rotation of the highly deformed nuclei which exist there; there is some well established evidence for these deformations from nuclear quadrupole moment measurements, which are a direct index of the departure from the spherical shape in these nuclei. This rotation leads to energy levels whose position is given by the simple quasi-molecular expression

$$E_I = \frac{\hbar^2}{2\mathfrak{J}} I(I+1), \quad (3)$$

where *I* is the angular momentum (spin) of the level, and  $\mathfrak{J}$  is the effective moment of inertia of the nucleus (which is *not* the actual moment of inertia, but only that part of it which is associated with the deformation from sphericity). For nuclei containing an even number of both protons and neutrons (even-even), the theory predicts a level sequence: *I*=0, 2, 4, 6,

. . . , all with even parity. For nuclei of odd mass number, the sequence predicted is  $I_0, I_0+1, I_0+2, \dots$ , where  $I_0$  is the (half-integer) ground-state spin; all levels have the same parity as the ground state. Because of the selection rule mentioned above, we see that according to this picture, we should excite but one level in even-even nuclei having spin  $2^+$ ; in the odd

rather remarkable agreement was found with these predictions. In some of these cases, final confirmation of the correctness of the level assignments must await the availability of enriched isotopes of some of the rare earths, where many isotopes exist in a given element.

Confirmation of the correctness of the proposed level schemes has been obtained

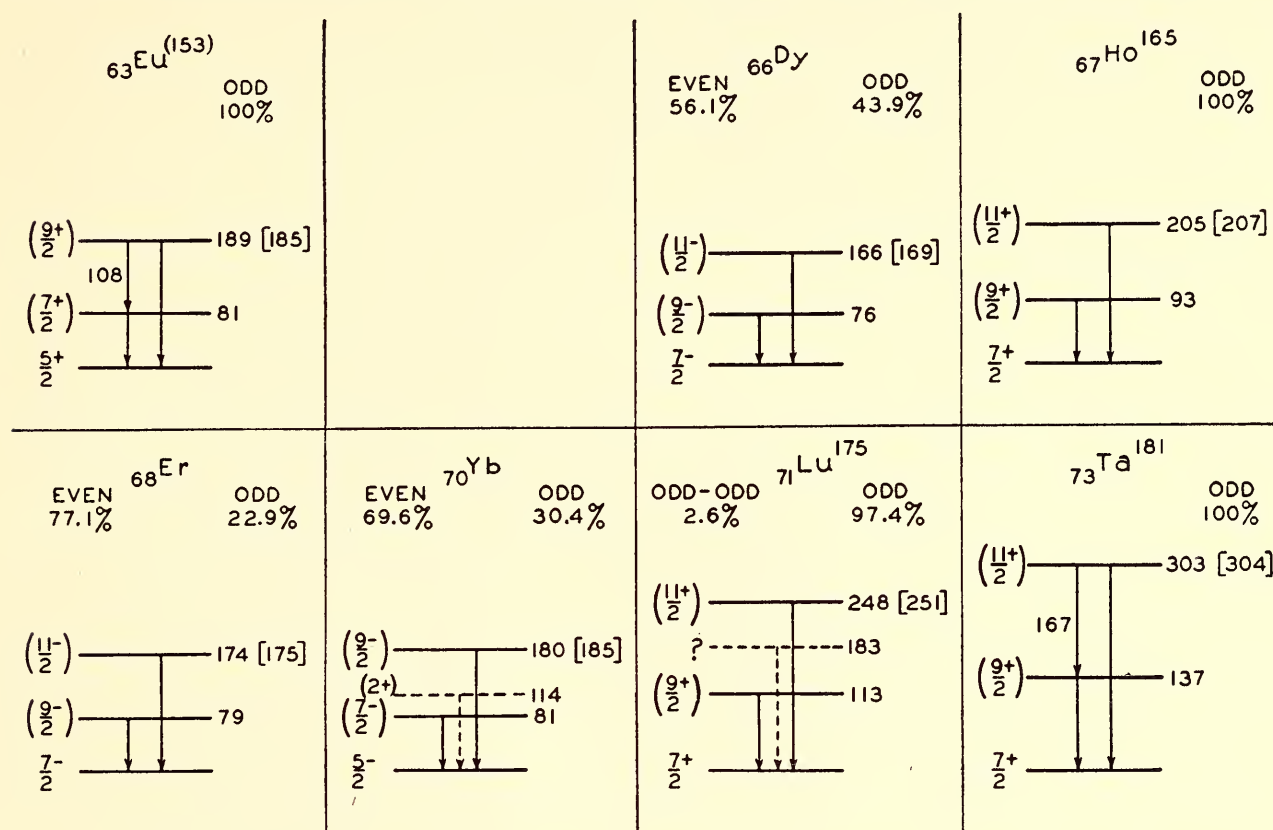


FIG. 11. Energy-level diagrams for rotational levels in the rare-earth nuclei and in tantalum. Numbers in brackets are the energies (in kev) predicted from positions of first excited states; all other values are observed energies in kev.

nuclei, we should excite the first *two* excited states. It is clear that the odd nuclei make it possible to check the theory merely by comparing predicted and observed level spacings. The absolute value of the position of the first excited state depends on the quantity  $\mathfrak{J}$ , and must be taken from experiment; once this is known, one can uniquely predict the position of the higher excited states. For example, if the ground state has spin  $7/2$ , the first excited state will have spin  $9/2$ , and the second excited state, with spin  $11/2$ , will be located at 2.22 times the energy of the first level. Figure 11 presents a summary of the cases where

in the cases of tantalum and europium by observing coincidences between the two cascading gamma rays from the upper level.

An interesting exception to the regular rotational pattern in odd nuclei occurs in the case of spin  $1/2$  in the ground state. We have studied three of these nuclei, rhodium-103, silver-107, and silver-109, and found two hitherto unknown levels in each case. We illustrate the case of the two silver isotopes in figure 12. Their similarity in all respects is rather striking. The appropriate parameters introduced by the collective theory derived from all three of



these nuclei are very nearly the same and bear out the rotational interpretation.

In the even-even nuclei, as was already mentioned, we see but one level, and if we see it its spin must be  $2^+$ , since the ground states are always  $0^+$ . Some confirmation for the positions of higher levels in these nuclei comes from alpha-ray spectroscopy of the heaviest elements; in some cases the sequence has been seen up to  $8^+$ . Furthermore, there are unique predictions con-

number of doubly charged helium ions is being accelerated in our machine (to twice the energy, or up to 7 Mev) to permit effective experiments on Coulomb excitation. The cross section is such a steep function of bombarding energy that the loss in current is more than offset by a gain in yield. We have already been able to see excited states in lighter nuclei up to 1 Mev. This makes it possible to find levels in even-even nuclei (which are located between 500 kev and 1 Mev at the low end of the periodic table) and immediately assign their spins and parities. Considerable effort is required to do as much by conventional radioactivity techniques (angular correlations, internal conversion measurements, and the like).

Aside from levels bearing out the Bohr-Mottelson picture, we see a number of either previously known or new energy levels with smaller transition probabilities ("single-particle" transitions), mainly in the lighter nuclei. In several cases we were able to choose definitely from among a number of decay schemes permitted from nuclear spectroscopy measurements. For instance, we can always tell which of two gamma rays is lower in a cascade transition, whereas coincidence measurements will in general not allow such a choice to be made.

Coulomb excitation by alpha particles, then, turns out to be an extremely versatile tool in nuclear spectroscopy, for nuclei from  $Z=9$  to  $Z=92$ , and has already shed considerable new light on transitions between low-lying energy levels.

#### DISINTEGRATION OF LIGHT NUCLEI BY ALPHA PARTICLES

During the past year we have continued our study of light-element disintegration by alpha particles. Of interest here has been the type of reaction which can occur with alpha particles of 3.5 Mev energy, and the resonance structure of the yield of the particles emitted, which then gives information about the energy levels in the compound nucleus.

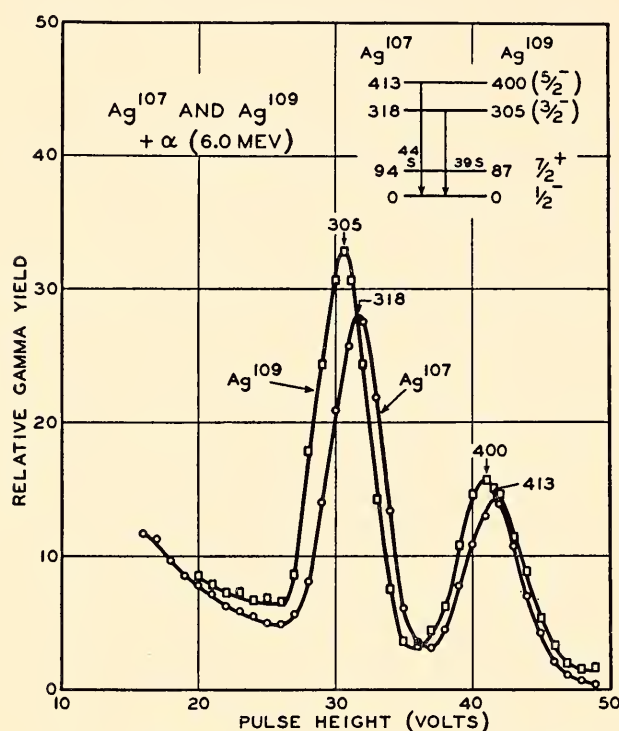
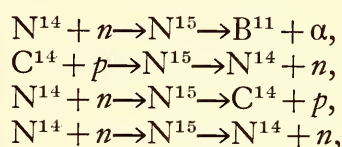


FIG. 12. Pulse-height distributions for gamma rays from the two silver isotopes  $\text{Ag}^{107}$  and  $\text{Ag}^{109}$  bombarded by 6-Mev alphas. The energy-level scheme is given, showing the similarity between the two isotopes.

cerning the actual strengths of these rotational transitions, in terms of the same parameters which describe the nuclear nonsphericity. Within the experimental uncertainties, these predictions have been verified in most cases. The rotational character of these levels is hard to refute in view of this evidence. Many refinements in the experiments are required to confirm details of the theory, such as the spin assignments. Some workers have already found agreement in a few cases.

We have recently found that a sufficient

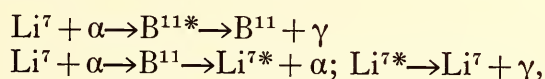
The neutron yield has been measured as a function of the alpha particle energy for the reaction  $B^{11} + \alpha \rightarrow N^{15} \rightarrow N^{14} + n$ , where  $\alpha$  and  $n$  are symbols for alpha particles and neutrons. The neutrons were detected with a boron trifluoride proportional counter (enriched in  $B^{10}$ ) at right angles to and along the direction of the incident alpha beam. With thin evaporated boron targets, the neutron yield showed a pronounced resonance structure. Resonance peaks occurred at alpha energies 1.28, 1.36, 1.54, 1.59, 1.87, 2.09, 2.47, 2.63, 2.99, and 3.26 Mev; these correspond to energy levels in the compound nucleus  $N^{15}$  at 11.91, 12.00, 12.11, 12.16, 12.35, 12.51, 12.76, 12.92, 13.19, 13.38 Mev, respectively. This energy region in  $N^{15}$  has been investigated by four other reactions involving  $N^{15}$  as a compound nucleus, at other laboratories, namely:



where  $p$  is the symbol for a proton.

All levels of  $N^{15}$  which had previously been observed are included in the list given above from our observations; in addition, we found a level not previously observed.

The gamma-ray yield has been investigated for the following two reactions with lithium:

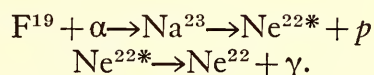


where  $\gamma$  is the symbol for gamma radiation.

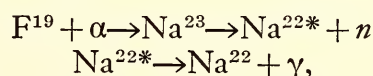
The gamma radiation was detected with the scintillation spectrometer used in the Coulomb excitation study. The first of these reactions is an alpha capture process. There are three known resonances in  $B^{11}$  at alpha energies of 0.40, 0.82, and 0.96 Mev. The highly excited  $B^{11}$  nucleus is known to return to its ground state primarily by gamma rays cascading through a level at 4.5 Mev. A search was made for further resonances with alphas up to 2.5 Mev, and none were found. Above 1 Mev,

478-keV gamma radiation was observed, which results from the excitation of the first excited state of  $Li^7$  by inelastic scattering of alpha particles. The yield curve for the 478-keV gamma ray exhibits three broad resonances at energies 1.91, 2.46, and 3.06 Mev. From these resonances the calculated energy levels in  $B^{11}$  are at 9.88, 10.24, and 10.62 Mev, respectively.

Fluorine is the lightest element for which low-lying levels are Coulomb-excited by alpha particles. The yields of both the 113- and the 196-keV gamma rays from the first and second excited states in  $F^{19}$  agree with the predicted yield for Coulomb excitation up to alpha energies of about 2.0 Mev. It is known from lifetime measurements for these two levels (from work done by the group at California Institute of Technology) that the first level is excited by electric dipole radiation and the second by electric quadrupole radiation. Above 2 Mev, resonances appear in increasing strength as the alpha energy is increased. These resonances are due to energy levels in the compound nucleus  $Na^{23}$ . A gamma ray, having an energy of 1.28 Mev, was also observed from fluorine. The following reaction is responsible for this gamma radiation:



The first excited level of  $Ne^{22}$  occurs at 1.28 Mev. Weak resonances are observed well below the alpha energies at which the 113- and 196-keV gammas are excited predominantly by the Coulomb excitation process. A fourth gamma ray of energy 592 keV appeared for alpha energies greater than 3.05 Mev. We surmised this gamma to result from the reaction



where the  $Na^{22}$  nucleus is left in its first excited level at 592 keV, and decays to its ground level with the emission of a 592-keV gamma ray. The reaction in which no gamma ray is emitted, i.e.  $Na^{22}$  is left in its ground level, should have a threshold



of 2.3 Mev, calculated from the masses of the particles, below which the reaction cannot occur. We searched for neutrons and found a threshold at close to the predicted energy. Above the threshold, the neutron yield showed the resonance structure characteristic of the compound nucleus  $\text{Na}^{23}$ . The 592-keV gamma ray from the above reaction should then have a threshold at

ties, and the corresponding energy levels in  $\text{Na}^{23}$  for the four gamma rays from fluorine.

Sodium is another nucleus for which both Coulomb excitation and compound nucleus formation were observed with alpha-particle bombardment. The first excited level in  $\text{Na}^{23}$  occurs at 446 keV. This level is excited by inelastic alpha scatter-

TABLE 6  
ENERGY LEVELS IN  $\text{Na}^{23}$

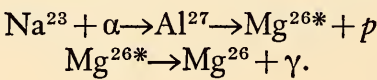
$E_c$  is the excitation energy of the compound nucleus  $\text{Na}^{23}$ . Columns 3, 4, 5, and 6 give the relative resonant yields of the various competing outgoing channels. Each gamma-ray yield is independently normalized.

| $E(\text{LAB})$<br>(MEV) | $E_c$<br>(MEV) | RELATIVE INTENSITIES OF GAMMA RAYS      |   |                                   |                                    |
|--------------------------|----------------|---|---|-----------------------------------|------------------------------------|
|                          |                | 113 keV<br>( $\alpha, \alpha' \gamma$ ) | 196 keV<br>( $\alpha, \alpha' \gamma$ ) | 592 keV<br>( $\alpha, n \gamma$ ) | 1.28 MeV<br>( $\alpha, p \gamma$ ) |
| 1.49.....                | 11.73          | ...                                     | ...                                     | ...                               | 0.04                               |
| 1.70.....                | 11.91          | ...                                     | ...                                     | ...                               | 0.02                               |
| 1.91.....                | 12.08          | ...                                     | ...                                     | ...                               | 0.8                                |
| 2.00.....                | 12.15          | ...                                     | ...                                     | ...                               | 0.4                                |
| 2.11.....                | 12.24          | ...                                     | ...                                     | ...                               | 0.5                                |
| 2.22.....                | 12.32          | 0.4                                     | 0.4                                     | ...                               | 2.8                                |
| 2.30.....                | 12.40          | ...                                     | 0.5                                     | ...                               | 0.8                                |
| 2.38.....                | 12.47          | ...                                     | 0.5                                     | ...                               | 2.2                                |
| 2.48.....                | 12.55          | 1.3                                     | 0.8                                     | ...                               | 6.8                                |
| 2.55.....                | 12.60          | 9.4                                     | 1.0                                     | ...                               | 11                                 |
| 2.64.....                | 12.68          | 11.4                                    | 4                                       | ...                               | 7.5                                |
| 2.76.....                | 12.78          | 3.2                                     | 9                                       | ...                               | 15.5                               |
| 2.84.....                | 12.85          | ...                                     | ...                                     | ...                               | 12                                 |
| 2.90.....                | 12.90          | 6.5                                     | 3                                       | ...                               | 17                                 |
| 3.02.....                | 13.00          | ...                                     | 12                                      | ...                               | 5                                  |
| 3.06.....                | 13.03          | 17.5                                    | ...                                     | threshold                         | ....                               |
| 3.18.....                | 13.13          | 149                                     | 12                                      | 3.5                               | 22                                 |
| 3.34.....                | 13.26          | 19                                      | 55                                      | 13                                | 20                                 |

3.05 MeV, just as observed. The energy value for the first excited level of  $\text{Na}^{22}$  is of interest in the study of the systematics of nuclei having an odd number of protons and odd number of neutrons, the odd-odd nuclei.  $\text{Na}^{22}$  was predicted to have its first excited state at about this energy. The level is also expected to have a rather long lifetime, of the order of 0.06 sec. We were able to set an upper limit of 0.01 sec on the lifetime, by an attempt to count gamma quanta after shutting off the alpha beam from the target.

Table 6 summarizes the results on the resonance energies, their relative intensi-

ties, and the corresponding energy levels in  $\text{Na}^{23}$  for the four gamma rays from fluorine. Sodium is another nucleus for which both Coulomb excitation and compound nucleus formation were observed with alpha-particle bombardment. The first excited level in  $\text{Na}^{23}$  occurs at 446 keV. This level is excited by inelastic alpha scatter-



Some sixteen resonances were observed for this gamma ray for alpha energies between 1.9 and 3.6 Mev. These resonances correspond to levels in the compound nucleus  $Al^{27}$ . The alpha energies at resonance and the level energies are listed in table 7. The contrast between the excitation curves for the two gammas is shown in figure 13. A weak gamma ray with energy 2.8 Mev was also observed by photographing the oscillo-

through a hole in the magnet yoke, allowing a path length of only 6 feet to the wall. This change will give considerably more room for external-beam experiments and will make it possible to have a shielded target space in the room. The cyclotron has been in operation since February. The external-beam intensity just beyond the aluminum window on the gate box has

TABLE 7

LEVELS IN THE COMPOUND NUCLEUS  $Al^{27}$  AS OBTAINED FROM  $Na^{23}(\alpha,p\gamma)Mg^{26}$ , FOUND IN THE 1.83-MEV GAMMA-RAY YIELD

$E_r$ , resonance energy in the laboratory system;  
 $E_{cm}$ , resonance energy in the center-of-mass system;  $E^*$ , excitation energy of  $Al^{27}$

| $E_r$     | $E_{cm}$ | $E^*$ |
|-----------|----------|-------|
| 1.95..... | 1.66     | 12.08 |
| 2.15..... | 1.83     | 12.24 |
| 2.43..... | 1.98     | 12.40 |
| 2.51..... | 2.14     | 12.56 |
| 2.56..... | 2.18     | 13.60 |
| 2.64..... | 2.25     | 12.67 |
| 2.80..... | 2.39     | 12.79 |
| 2.90..... | 2.47     | 12.87 |
| 3.04..... | 2.59     | 13.01 |
| 3.07..... | 2.62     | 13.04 |
| 3.16..... | 2.69     | 13.11 |
| 3.23..... | 2.75     | 13.17 |
| 3.29..... | 2.80     | 13.20 |
| 3.40..... | 2.90     | 13.32 |
| 3.50..... | 2.98     | 13.40 |
| 3.58..... | 3.05     | 13.47 |

scope trace of the gamma-ray spectrum. By this method it is possible to find weak gammas which would require very long counting times to be detected. This gamma ray results from the excitation of the second level of  $Mg^{26}$ .

The cyclotron has had some further alterations during the year. The entire vacuum system, including the dee box and dee stems, was rotated through an angle of 30° with respect to the magnet. This was done so that the external beam would clear the magnet yoke and allow a path length of about 25 feet to the corner of the cyclotron room. Originally the external beam was directed in such a way as to pass

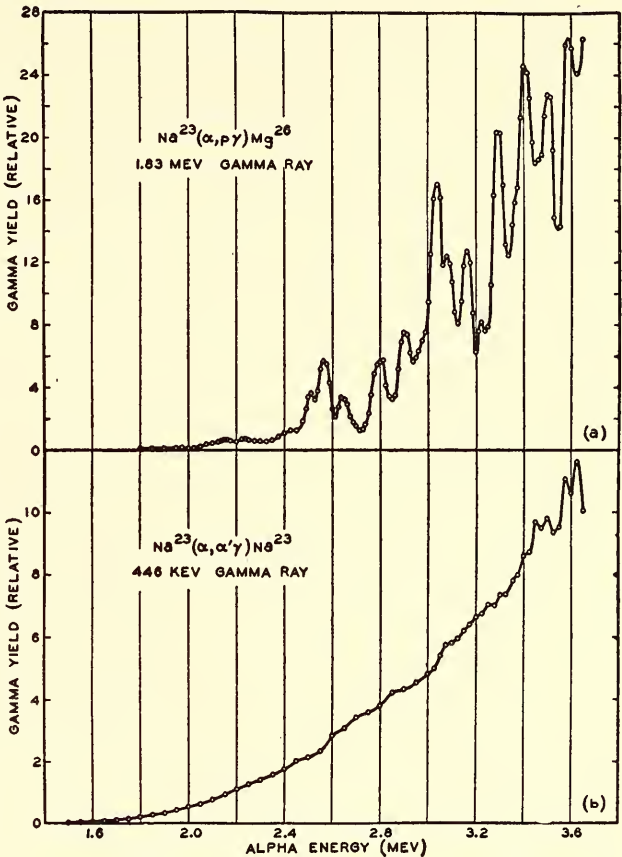


FIG. 13. Excitation curves of the 1.83-Mev (from  $Na^{23}(\alpha,p\gamma)Mg^{26}$ ) and the 446-keV gamma rays (from  $Na^{23}(\alpha,\alpha'\gamma)Na^{23}$ ) from a thin  $Na^{23}$  target, bombarded by alpha particles.

been 10 microamperes for deuterons and 0.5 microampere for alpha particles. The cyclotron has been used on a part-time basis for making radioactive targets for use in nuclear spectroscopy studies, some for our own work and a number for groups at the National Bureau of Standards.

During the year the upper one-third of the accelerating tube for the 3.5-Mev electrostatic generator was replaced with sections of improved design. The operation of the machine has been improved, and the remainder of the old tube will be replaced



when all the porcelain sections have been received.

### NUCLEAR ALIGNMENT

In co-operation with Drs. E. Ambler and R. P. Hudson of the National Bureau of Standards, Temmer has carried out at the Bureau of Standards a nuclear alignment experiment at very low temperatures, making use of the so-called crystalline hyperfine-structure method of alignment proposed by Bleaney and carried out at Oxford and Leiden for cobalt-60. We have used the radioactive nucleus cerium-141. The experiment is carried out with a single crystal of cerium-magnesium nitrate containing some of the radioactive material. By adiabatic demagnetization it is possible to reach temperatures of about  $0.003^\circ \text{K}$  with this salt. At this temperature the splitting between the nuclear magnetic substates is of the order of  $kT$ , and the lowest levels have the highest populations. The magnetic field acting on the nuclear magnetons is that produced by the paramagnetic electrons through the hyperfine interaction. It is peculiar to the Bleaney nuclear alignment method that positive and negative values of the magnetic quantum number  $m$  are equally populated, whereas in a method of nuclear polarization (Gorter and Rose), an external residual field serves to preserve a sense of direction by orienting the electron spins. For radioactive measurements of the type described here, however, alignment is sufficient, since the polar diagrams for gamma-ray emission are always symmetric about the equatorial plane. In the particular case of cerium-magnesium nitrate, the alignment is unusual in that it occurs in a plane rather than along an axis.

The measurement consists in determining the anisotropy of the gamma radiation emitted from this nucleus (following its unobservable beta decay) as a function of the temperature, which is simultaneously measured by standard magnetic susceptibility techniques. Figure 14 shows the experimental set-up, which consists of two scintillation counters viewing the crystal

sample contained in the double Dewar vessel, along and at right angles to the preferred crystal axis. The counting rates were found to differ by 13 per cent at the lowest temperatures, and to approach equality with the warming up of the sample, the difference being essentially zero by the time the temperature reaches  $0.1^\circ \text{K}$ , which, in a typical run, takes about 5 to 10 minutes. The data obtained are summarized in figure 15. From the anisotropy it was possible to reach the following con-

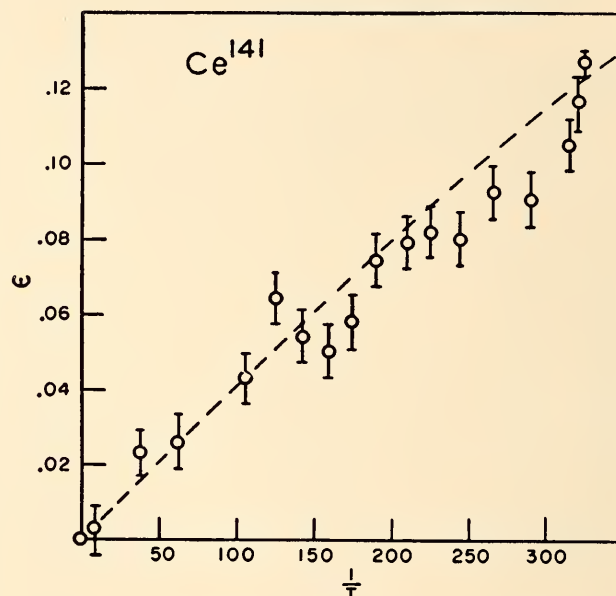


FIG. 15. Summary of results on alignment of  $\text{Ce}^{141}$ . Plot shows the anisotropy  $\left( \frac{\text{counting rate B} - \text{counting rate A}}{\text{counting rate B}} \right)$

as a function of the reciprocal absolute temperature. The point of highest anisotropy (with smallest statistical error) is the only one needed to reach our conclusions.

clusions concerning the decay of cerium-141; (a) the gamma radiation is predominantly of the magnetic dipole type, whereas either magnetic dipole or electric quadrupole radiation was admitted from previous evidence; (b) the lifetime of the excited state must be less than  $10^{-10}$  seconds for this amount of anisotropy to persist (no loss of memory in the intermediate state); (c) the magnetic moment of the nucleus cerium-141 is about 0.3 nuclear magnetons. These measurements cannot now be obtained in any other way.



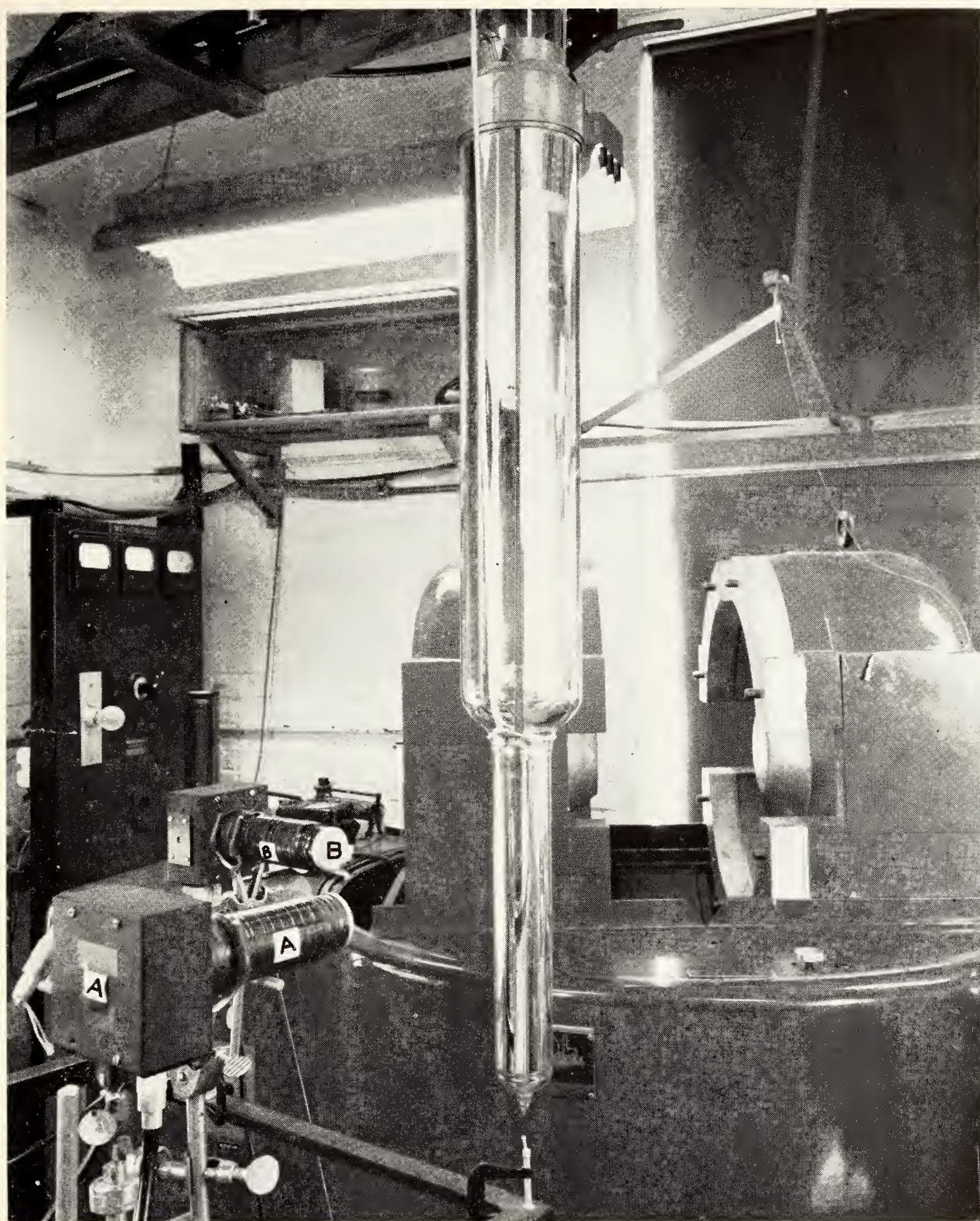


FIG. 14. Experimental set-up for the nuclear alignment experiment, showing the two scintillation counters (*A*, *B*) viewing the radioactive crystal sample contained in the double Dewar vessel. The magnet providing the 23,000-gauss magnetic field used in the adiabatic demagnetization is shown in the background. Counter *A* lies along the normal to the alignment plane; counter *B* lies in the alignment plane.





Very recently a further alignment experiment was carried out successfully with the same salt containing, in addition, radioactive neodymium-147 with a half life of 11 days. One gamma ray (91 kev) was found to be isotropic and another was found to have about 30 per cent anisotropy, indicating quadrupole radiation. The isotropy of the first gamma radiation implies a magnetic dipole-electric quadrupole mixture in about equal proportions.

A number of targets were bombarded with the Department's cyclotron in an effort to find other suitable nuclei for alignment. This field is extremely limited at present, mainly because of the many solid-state requirements which have to be met when the Bleaney method is used.

### BIOPHYSICS

P. H. ABELSON, E. T. BOLTON, R. J. BRITTEN,  
D. B. COWIE, AND R. B. ROBERTS

The past year has marked a period of transition for the biophysics group. We have attempted to shift the direction of our work from the study of the biosynthesis of amino acids and nucleic acid bases toward a study of synthesis of the macromolecules. Several exploratory ventures were carried out in this field, including studies of the role of peptides in protein synthesis, the kinetics of protein and nucleic acid synthesis, and the incorporation of tracers by cell fragments. In addition, considerable progress was made in learning how the components of nucleic acid are assembled.

It has been difficult, however, to break away completely from the previous studies of amino acid synthesis. A large share of the available time was spent in the preparation of a monograph describing previous work with the smaller molecules. Many experiments had to be carried out to provide additional data required for the monograph. Some of these experiments showed unexpected features which required further investigation.

### STUDIES OF ACETATE-ADAPTED CELLS

In most of our studies of amino acid synthesis, glucose was used as the energy source. With this source, the Krebs cycle is used by the cell chiefly to synthesize amino acids, and provides little energy. If the cells use acetate as their carbon source, however, energy is not available as before from the degradation of glucose to acetate; on the contrary, energy must be supplied to reverse the usual reactions of degradation and to build up the more complicated molecules from acetate. In this situation the cells are forced to utilize the reactions of the Krebs cycle for their supply of energy.

Such general considerations, however, could not determine the details of the reactions by which acetate entered the Krebs cycle. Furthermore, there has been a consistent difficulty in observing competition by the postulated intermediates of glycolysis when glucose was used as carbon source, and the origin of several amino acids could not be defined. Experiments were therefore carried out using acetate-adapted cells.

As a first step  $C^{14}H_3COONa$ ,  $CH_3C^{14}OONa$ , and  $C^{14}O_2$  were used as tracers in parallel cultures growing with acetate and  $CO_2$  as the sole carbon sources. The specific radioactivities of the amino acids showed clearly that acetate enters the Krebs cycle by two routes. In one of these, acetate condenses with  $CO_2$  to form a three-carbon unit (probably phosphoryl pyruvate) which then condenses with a second molecule of  $CO_2$  to form oxalacetate, which participates in the usual reactions of the Krebs cycle. Acetate also enters the cycle directly at a rate 5 to 10 times as high as in glucose-utilizing cells, and its oxidation in passing around the cycle provides the required energy. In contrast with the situation observed when glucose is the energy source, the flow circulating around the cycle is large as compared with the flow leaving the cycle as finished products of synthesis.



In addition to the usual flow from the cycle to supply aspartic acid and glutamic acid, the Krebs cycle of acetate-adapted cells also provides three-carbon fragments (pyruvate or lactate). These three-carbon units are then utilized by the cell in the synthesis of other amino acids (fig. 16). The differences and the common features of the glucose and the acetate metabolism are indicated in figures 16 and 17. In general, the same reactions are used but the

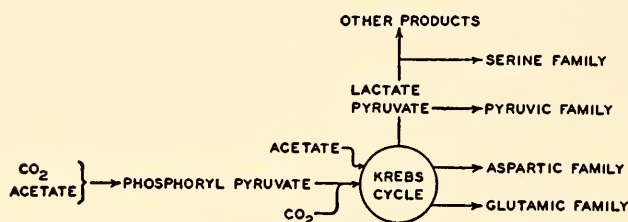


FIG. 16. Flow of carbon from acetate

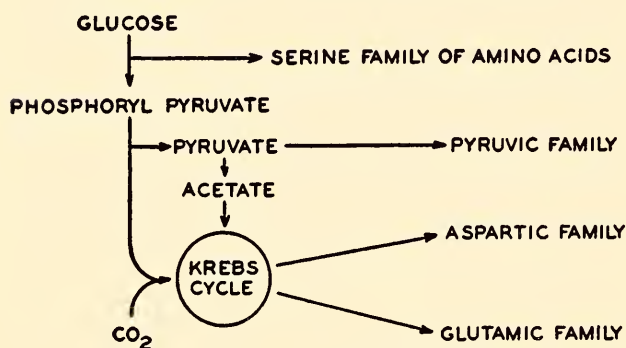


FIG. 17. Flow of carbon from glucose

flow rates are altered to meet the new situation.

As acetate is a compound of relatively low free energy, its products must be "pumped uphill." In this situation many intermediates of glycolysis which are ignored by the cell when glucose is present are rapidly incorporated when acetate is the only alternative. Fructose-6-phosphate,  $\alpha$ -glycerolphosphate, and phosphoglyceric acid have no effect on the incorporation of glucose carbon, but cause a marked reduction in the incorporation of acetate carbon. In particular, pyruvate, which supplies only 60 per cent of the carbon of valine when glucose is available, was found to supply 96 per cent when acetate was the alternative. Glycine provides no carbon for serine to cells growing on glucose, but

provides 95 per cent of the serine carbon in cells growing on acetate.

The glycine situation is particularly interesting, as it shows a reversal of the usual serine  $\rightarrow$  glycine reaction. The same reversal was observed when fructose replaced glucose as the energy source. There is a marked difference between fructose and glucose as sources of carbon for serine synthesis, but according to the conventional reactions of glycolysis these two sugars should be metabolized quite similarly.

We are therefore led to search for some quite different mode for the synthesis of serine from glucose. Furthermore, the cells have a very active serine deaminase which converts serine to pyruvate. Such a pathway could lead directly from glucose through serine to pyruvate, by-passing the usual reactions of glycolysis, also by-passing the many difficulties which we have found in interpreting our results in accordance with the conventional scheme of glycolysis.

#### NITROGEN METABOLISM

Until this year the investigations of biosynthesis have been largely concerned with the flows of carbon and sulfur in the cell, and only a few observations have been made on nitrogen incorporation. No description of synthesis is adequate, however, until the main features of nitrogen incorporation have been uncovered. A beginning in this direction was made during the report year in collaboration with Drs. R. D. McAfee and R. T. Nieset of Tulane University, who carried out the isotope analysis of samples prepared here.

The study of nitrogen metabolism is much more difficult than a comparable study of carbon, because the radioactive isotopes of nitrogen have lifetimes too short for biological work. Consequently, it is necessary to use the stable isotope  $N^{15}$  as a tracer. Unlike the measurements of radioactivity, the mass spectrographic determinations of isotope ratios call for con-

siderable quantities of nitrogen and require that the various compounds be converted to nitrogen gas. As the quantity of nitrogen available for analysis is diminished, the errors due to contamination of the samples increase. A quantity of 10  $\mu\text{M}$  can be analyzed, but more is desirable. The routine separation of amino acids by paper chromatography is not satisfactory, since the maximum quantity of protein hydrolyzate giving good resolution yields only 0.5 to 1.0  $\mu\text{M}$  of each amino acid. By overloading the paper and sacrificing the resolution, yields of 1.5 to 3  $\mu\text{M}$  can be obtained. It is therefore difficult, though possible, to carry out competition experiments with  $\text{N}^{15}\text{H}_4^+$ .

In view of these difficulties, nitrogen metabolism was explored by several indirect methods to minimize the number of  $\text{N}^{15}$  experiments needed. The  $\text{N}^{15}$  work is in the very early stages and requires repetition and further extension. Some of the findings, however, have already been useful in the interpretation of the carbon results, and can be reported.

*Growth with various nitrogen sources.* A number of amino acids were tested as sole nitrogen sources. Cells were taken from cultures growing exponentially, centrifuged, washed, and resuspended in a medium lacking  $\text{NH}_4^+$ . Amino acids were added at a concentration of 4  $\mu\text{M}$  per ml, and the resulting growth was measured. DL-Serine, DL-aspartic acid, and DL-alanine gave the best growth among the compounds tested, the growth rate being approximately the same as with  $\text{NH}_4^+$ . The growth rate with L-glutamic acid was one-half the control, and methionine, valine, glycine, threonine, adenine, uracil, and cytosine gave slower growth rates, ranging from one-third to one-fifth of the  $\text{NH}_4^+$  control. D-Glutamic and L-proline showed no growth at first, but the cells finally became adapted to the use of proline after several days of incubation.

With the other amino acids there was no evidence of adaptation except for a slightly

reduced growth rate during the first half hour.

As the pH of the medium has an influence on the formation and the activity of deaminating enzymes, glutamic acid and aspartic acid were tested as sole nitrogen sources under various conditions of pH. Cells grown at pH 6 could not be distinguished from cells grown at pH 7. With aspartic acid as the sole nitrogen source, the growth rate was 1.5 times as high at pH 7 as at pH 6. With glutamic acid or  $\text{NH}_4^+$  as the nitrogen source, there was no difference in growth rate between pH 6 and pH 7.

*Sparing action.* In the experiments dealing with sulfur sources it was found that although methionine cannot supply all the sulfur requirements of the cell, it does supply one-half the sulfur and consequently shows a considerable sparing action on the use of sulfate. Some of the amino acids were tested for their effectiveness in sparing the use of  $\text{NH}_4^+$ .

These tests were carried out in two ways. In one method, 2  $\mu\text{M}$  per ml of  $\text{NH}_4^+$  was added to the medium. One culture served as control, and the others had 4  $\mu\text{M}$  per ml of various amino acids added as supplementary sources of nitrogen. When the  $\text{NH}_4^+$  was exhausted, the growth rate shifted abruptly to that characteristic of growth using the additive as a sole nitrogen source. The breaks in the curves thus indicated the sparing action of the additive. This method has the advantage of being simple and easy, thus allowing a rapid survey of a large number of nitrogen sources.

The second method consisted in measuring the residual  $\text{NH}_4^+$  after a period of growth in the presence of  $\text{NH}_4^+$  and the additive.

Alanine, aspartic acid, glycine, glutamic acid, isoleucine, serine, and valine were tested by these methods. The results were completely in accord with the previous tests using the amino acids as sole sources. The amino acids which gave the highest growth rates as sole sources also showed



the greatest sparing action. Thus no separate and distinct points of entry or utilization of nitrogen were uncovered.

These measurements indicated that roughly 16 mM of  $\text{NH}_4^+$  was required for the growth of one gram dry weight of cells. Direct measurements of the nitrogen content of the cells agree with calculations from the composition of the cells in showing that the content of the cells is 11 mM per gram. The difference between these two values indicates that some nitrogenous waste products have been produced and released into the medium.

The corresponding rates of nitrogen metabolism for the standard generation time of one hour are: 210  $\mu\text{M}$  per g per 100 sec incorporated into the cells, and 305  $\mu\text{M}$  per g per 100 sec of  $\text{NH}_4^+$  used.

When either aspartic acid or alanine is added to the medium, the rate of disappearance of  $\text{NH}_4^+$  is reduced by a factor of 2. When serine is present, the quantity of  $\text{NH}_4^+$  actually increases with time. The rates at which these amino acids supply nitrogen can be estimated to be approximately 150  $\mu\text{M}$  per g per 100 sec for alanine and aspartic acid and 500  $\mu\text{M}$  per g per 100 sec for serine. The high rate of deamination of serine shows why it is such a good nitrogen source, and explains the previously observed utilization of carbon from exogenous serine for the synthesis of compounds derived from pyruvic acid.

These measurements do not distinguish between deamination and transamination, and were, therefore, only preliminary to the use of  $\text{N}^{15}$ .

One of the simplest measurements with  $\text{N}^{15}$  is the isotope dilution of the medium. If nitrogen is incorporated from  $\text{N}^{14}$  aspartic acid by transamination, then the  $\text{N}^{15}\text{H}_4^+$  of the medium will not be diluted by  $\text{N}^{14}\text{H}_4^+$  even though the  $\text{N}^{14}$  aspartic acid has a considerable sparing action. On the other hand, if the  $\text{N}^{14}$  aspartic acid is deaminated, the  $\text{N}^{14}\text{H}_4^+$  so produced will exert a sparing action, but will at the same time dilute the  $\text{N}^{15}\text{H}_4^+$  of the medium. Such measurements show a high dilution

of the  $\text{N}^{15}\text{H}_4^+$  in the medium when  $\text{N}^{12}$  serine is present, but a much smaller dilution when  $\text{N}^{14}$  alanine or  $\text{N}^{14}$  aspartic acid is present.

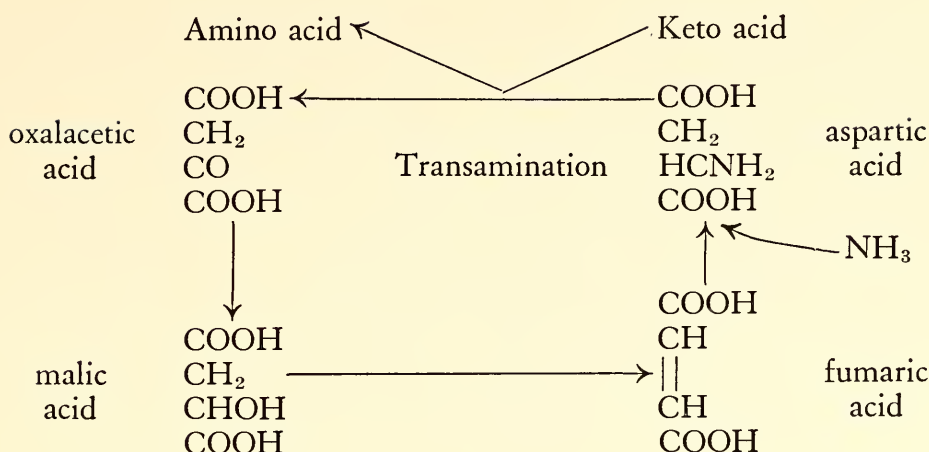
The isotope ratios in the various cellular fractions show the effects of transamination in addition to the deamination shown above. When cells grow in the presence of  $\text{N}^{15}\text{H}_4^+$  and  $\text{N}^{14}$  aspartic acid, the isotope ratios found in all the fractions of cellular compounds show that more than one-half the nitrogen is derived from aspartic acid. It is, moreover, derived by processes equivalent to transamination, since the  $\text{N}^{15}$  content of the medium is not appreciably diluted.  $\text{N}^{14}$  alanine causes a similar, but smaller, reduction in quantity of  $\text{N}^{15}\text{H}_4^+$  incorporated. The values obtained for the isotope ratios of individual amino acids are not yet reliable.

These few measurements give the impression that aspartic acid and alanine serve as important entry points of nitrogen. Even when  $\text{NH}_4^+$  is present, a large proportion of the cellular nitrogen is derived from these amino acids. In addition, some of the excess of exogenous amino acid is deaminated. When no  $\text{NH}_4^+$  is available, these amino acids can readily supply the total nitrogen requirement. It is a very reasonable guess that when  $\text{NH}_4^+$  serves as the sole source of nitrogen, a large proportion enters the cell by way of aspartic acid, alanine, and possibly glutamic acid. A favorable cycle to provide the energy required for incorporation of  $\text{NH}_4^+$  into aspartic acid exists (see diagram on p. 81).

There are indications of an analogous cycle involving alanine, pyruvic acid, lactic acid, and acrylic acid; and another analogous cycle involving glutamic acid,  $\alpha$ -ketoglutaric acid, hydroxyglutaric acid, and glutaconic acid is theoretically possible.

#### CELLULAR ORGANIZATION

The organization of the cell poses problems which tempt speculation. Many of the features of metabolism can be interpreted in terms of a simple model of the



cell, one in which metabolic intermediates diffuse freely from one active site to another within a region delimited by the cellular membrane. Such a model is helpful when metabolism is considered in a general way. The required compounds enter the cell from the medium and undergo reactions catalyzed by enzymes. A part of the material is used to supply energy, and waste products diffuse out of the cell. Another part is successively altered by a series of enzymes to provide the building blocks for protein and nucleic acid synthesis. These low-molecular-weight components then must diffuse from the enzymatic sites where they were formed to the multitude of different sites where the different types of proteins and nucleic acids are being assembled.

To operate efficiently according to this model, the cell would need a membrane which would allow the entry of raw materials such as glucose, ammonia, and phosphate and permit the release of waste products such as carbon dioxide. If metabolic intermediates were free to escape, the cells would have to synthesize intermediates for some time to build up a suitable concentration in the entire medium, before protein and nucleic acid synthesis could proceed.

Though this crude model is all that is necessary in considering the chemical changes which occur during metabolism, it is not adequate if free amino acids are assumed to be metabolic intermediates. Direct measurements have shown that the

cell wall presents no significant barrier to many molecules, including several amino acids. The whole usefulness of the competition method depends on the ability of exogenous competitors to reach the regions which cause alteration or incorporation into macromolecules. Furthermore, the study of extracellular products (Year Book No. 52) shows that amino acids such as glycine and glutamic acid stay within the cell or diffuse out into the medium depending on the concentration in the medium. Clearly, the cell is in intimate contact with its environment, and most if not all of the small molecules pass freely through the cell membrane.

We have seen no evidence, however, that any concentration of amino acids must be built up in the medium before protein synthesis can occur. When a growing culture is washed and inoculated into a fresh medium, its growth rate shows no alteration. Many cases have been found where the cells utilize an exogenous amino acid (even when this is present only in trace quantities) in quite different ways from endogenous material. In these cases it is certain that the material formed in the cell does not diffuse out into the medium and back before being used for protein synthesis.

Accordingly, we have here a paradox: amino acids are free to diffuse into and out of the cell, but in many cases they do not diffuse out. In a similar but perhaps less striking way, many compounds which seem to be intermediates (fructose-



6-phosphate is a good example) do not compete as might be expected. Other compounds which do diffuse inward and do compete still do not compete to the degree which would be expected on the basis of the simple model.

To resolve this paradox and to give a better interpretation of observations, the model of cellular organization must be revised. One possible revision is to assume that, even though the cellular membrane is permeable, certain regions of the cell are surrounded by impermeable or selectively permeable membranes. At first sight this hypothesis is attractive because it permits arbitrary limitations on the access of exogenous compounds to the reactive centers of the cell and provides a mechanism for holding endogenous metabolites within desired channels. When examined more critically, however, this hypothesis becomes untenable.

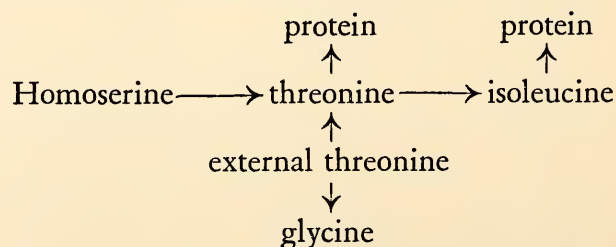
For example, if it is used to explain the behavior of threonine, it must be assumed that the region where threonine is synthesized, together with the regions where threonine is used for protein synthesis, is enclosed by a membrane of very peculiar properties. Exogenous threonine must be able to penetrate this membrane, as exogenous threonine can be utilized for protein synthesis. Threonine formed within the membrane must not be allowed to leak out, or it would encounter the enzymes which convert it to glycine. The region enclosed must include the entire group of reaction sites at which proteins are formed, because the threonine is needed at all of them. A similar region would be required for lysine, as the same distinction exists between endogenous and exogenous lysine. These two regions must be overlapping, since both threonine and lysine are required for protein synthesis. As such a situation is clearly absurd, the concept of internal permeability barriers must be discarded as a mechanism for keeping endogenous amino acids within the cell.

This model might have a limited applicability in interpreting some of the in-

stances in which suspected intermediates do not compete. For example, if some of the enzymes which carry out the early steps of glycolysis were wrapped in a membrane that was impermeable to hexose diphosphate, then hexose diphosphate would not be utilized by intact cells. Such membranes would also provide convenient packages for hiding some of the difficulties encountered in the Krebs cycle. Since, however, a different mechanism must be found to explain the transfer of amino acids from the site of synthesis to the sites of incorporation into proteins, it would seem more reasonable to discard the concept of small impermeable regions within the cell unless forced back to it by the experimental results.

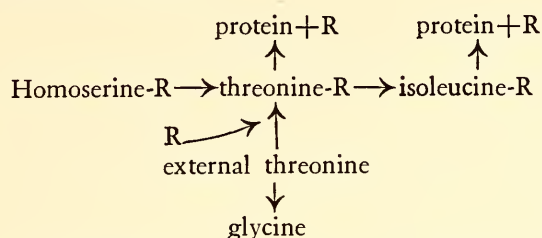
Most of the difficulties arise in attempting to explain why compounds supplied externally are not identical in behavior with endogenous compounds. These difficulties disappear as soon as it is realized that no proof exists that the exogenous and the endogenous compounds are in fact identical. Furthermore, there are many examples which show that endogenous material is carried by, or attached to, other molecules which may profoundly alter its detailed chemical behavior. Acetate is believed to be used as acetyl CoA and succinate as succinyl CoA. The phosphorylation of carbohydrates offers another example of a process where the alteration of the intermediates is not immediately apparent from a knowledge of the initial and final compounds.

Consequently, it seems quite reasonable to assume that most, if not all, of the intermediates of synthesis are in some altered form which can be indicated by attachment of a binding group, "R."



In the series of reactions diagramed above,

there is no apparent reason why endogenous threonine should not be converted to glycine. If an R group is attached, however, there is clearly a difference between the endogenous and exogenous threonine, as shown below:

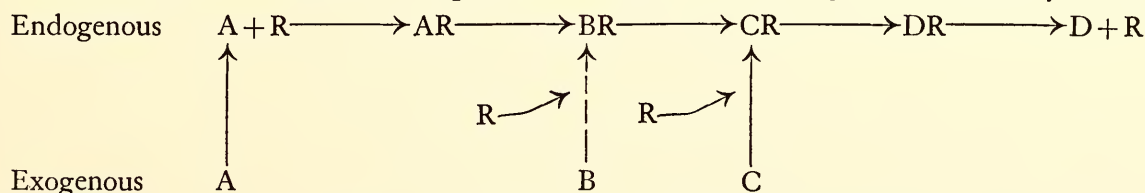


To interpret the metabolism of threonine, it is only necessary to assume (1) that the equilibrium conditions favor the formation of threonine-R and (2) that the enzyme which splits threonine to glycine cannot attack threonine-R.

The same concept is equally useful in interpreting the metabolism of other amino acids (that is, lysine and ornithine) in which the externally supplied amino acid is metabolized differently from the endogenous intermediate of protein synthesis. It is also necessary in the interpretation of some of the results obtained with sulfur.

Such an attachment also provides a mechanism whereby exogenous amino acids can diffuse freely through the cellular membrane but the amino acid intermediates of protein synthesis manufactured by the cell are not lost to the medium. According to this hypothesis, the R group must be large enough to prevent the outward diffusion of the metabolic intermediates.

Finally, the introduction of the R group provides an additional reaction and hence an additional free parameter in the quantitative interpretation of competition results. In the reaction series below, compounds



A and C might compete well in the formation of the final product D if the reactions  $\text{A} + \text{R} \rightarrow \text{AR}$  and  $\text{C} + \text{R} \rightarrow \text{CR}$  pro-

ceeded rapidly. At the same time compound B might compete poorly or not at all if the reaction  $\text{B} + \text{R} \rightarrow \text{BR}$  proceeded slowly.

Some definite examples of such reactions have been observed. Acetate is believed to be used as acetyl CoA ( $\text{Ac-R} = \text{Ac-CoA}$ ); exogenous acetate, however, enters the metabolic cycle without difficulty, showing that acetate is readily attached to CoA even though this reaction is endothermic. This reaction is similar to the first reaction  $\text{A} + \text{R} \rightarrow \text{AR}$ . On the other hand, external pyruvate does not enter the Krebs cycle, presumably because the active intermediate is not pyruvate but phosphorylenolpyruvate. This reaction is similar to the one where B (pyruvate) is not readily converted to BR (phosphorylenolpyruvate). Intermediate cases are also known where the external compound competes incompletely and the degree of competition does not depend in any simple fashion on the concentration of the exogenous compounds. Incomplete competition could be due either to a slow rate of association of the external material with the carrier R or to a slow rate of dissociation of the endogenous complex.

In short, the concept of altered intermediates is entirely adequate and often necessary to interpret the experimental results found in studies of permeability, extracellular products, and competition.

Unfortunately, there are very few hints as to the nature of the R groups involved. In some cases studies of separated enzymes have identified the group. Thus, CoA is seemingly the R group involved in acetate condensation, and in the reactions of succinate. Coenzyme-A is large enough so that it could quite reasonably hold the

metabolic intermediates within the cell membrane.

On the other hand, though phosphate



can be considered a part of the R group which is attached to glucose and fructose, it is not large enough to be the entire R group. The cell wall is permeable to the hexose phosphates. Perhaps the phosphate serves as a handle by which the carbohydrates are attached to larger carriers.

There is no direct evidence whatsoever as to the nature of any R groups concerned in amino acid synthesis. Most of the amino acids are good competitors, a fact which indicates a rapid exchange between the free and the bound forms. In bacteria which accumulate amino acids, an energy source (glucose) is required for net accumulation, but an exchange of the internally bound amino acids with external amino acids occurs even in the absence of glucose. The glucose requirement can be taken to indicate a possible energy storage in the bound forms which might be useful in forming peptide bonds.

As to the nature of the molecules involved as R groups, there are two limitations: the molecule should be large enough to keep the amino acids within the cellular membrane, but small enough to permit the amino acids to reach the sites where they are utilized in protein synthesis. Large peptides or even proteins are possibilities, in addition to smaller molecules such as coenzymes.

At present, however, by assuming that binding does occur it is possible to revert to the first simple model of the cell. According to this model the small molecules of the medium can diffuse into the cell and reach the reactive centers. At the same time, any small molecules free in the cell can diffuse out into the medium. During the course of the metabolic reactions of the cell, however, the intermediates are not free, but bound to some larger molecule. Accordingly they can diffuse from one reactive site to another, but they cannot diffuse out of the cell. The binding molecule also serves to limit the range of enzymatic sites available to the intermediates. Spatial organization of enzymes, as suggested by studies of mitochondria, may

well occur in these cells. In our own work there is no direct evidence for it, but only the indirect evidence that the cell is unbelievably efficient in carrying out a multitude of simultaneous reactions.

#### BIOSYNTHESIS OF NUCLEIC ACIDS IN *ESCHERICHIA COLI*

The nucleic acids have been important objects of research since their discovery nearly a century ago. But only in recent years have we witnessed rapid progress toward understanding their chemical structures, their biological function, and their biosynthesis. Very many facts are known about the biosynthesis of the purine and pyrimidine ring systems, the synthesis of the pentose structure, and the formation of the nucleotide substructures which comprise nucleic acid. The relatively simple materials which can serve living organisms as sources of the atoms of nucleic acid seem now to be well established; and the directions taken by the biochemical pathways which lead from the simple materials toward nucleic acid sub-units are also clearly charted.

Nevertheless, much yet remains to be done before the nucleic acid area of the "metabolic map" can properly be called a charted territory. It is not known, for example, just what sub-units enter nucleic acid macromolecules, or what kinds of processes link the sub-units together into strands. A number of theories have been suggested to account for nucleic acid formation, but it is yet too early to assess which of them are of central significance for the formation of nucleic acid by living organisms.

To bring these several topics into a larger community of meaning, one would like to know how some particular living organism assembles small molecules to yield nucleic acid macromolecules. The biophysics group therefore began a systematic study of nucleic acid synthesis in the bacterium *Escherichia coli*. This organism is a heterotrophic bacterium which grows

readily in simple, chemically defined culture media. At 37° C it gives rise to a new generation approximately each hour during exponential growth. It will utilize relatively simple carbon sources such as glucose and carbon dioxide to supply its carbon requirements. It will also utilize a host of supplemental carbon compounds, and it ordinarily makes use of each in a specific fashion.

The fragments of nucleic acid which have been our main concern are the purines adenine and guanine; the nucleotides of cytosine and uracil; and ribose derived from the purine nucleotides. Each of these components may be isolated by paper chromatography and estimated accurately by chemical and isotopic determinations. About 300 micrograms of nucleic acid are usually analyzed in this way. Deoxyribonucleic acid is, quantitatively, a minor component making up only about one-sixth of the total. For this reason it cannot easily be studied by the routine procedure just described. We have therefore devoted our initial attention to the synthesis of the bulk component, ribonucleic acid.

Table 8 summarizes some results of a typical experiment designed to measure the incorporation of CO<sub>2</sub>. The data are expressed in terms of radioactivity in counts per second contained in each micromole of the component isolated. They show that both of the purines and both of the pyrimidine nucleotides are labeled, and that the isotope is equally distributed between the members of a pair. No C<sup>14</sup> is found in the pentose. This sugar is derived from the original purine-nucleotide segment of the macromolecule. When the pyrimidine nucleotides cytidylic and uridylic acids are also chemically degraded to yield the free base, all the C<sup>14</sup> originally in the nucleotide is then found in the pyrimidine ring. Therefore, this straightforward experiment shows that the CO<sub>2</sub> supply of the culture medium serves as a source of carbon for the purine and pyrimidine rings in *E. coli*. It is also evident

that the pyrimidines are more highly labeled than the purines. Thus a difference in the modes of synthesis of the two classes of chemicals is indicated.

By shifting the C<sup>14</sup> label from one carbon compound to another in the basal culture medium, or by supplying the cells with other labeled materials, one at a time, and carrying through the routine of analysis, it is possible to explore a large area of nucleic acid biochemistry in a very short while. Table 9 gives some qualitative results of such an exploration. The labeled compounds supplied to the cells are shown

TABLE 8  
UTILIZATION OF C<sup>14</sup>O<sub>2</sub> FOR NUCLEIC ACID  
SYNTHESIS BY *E. COLI*

| Component               | Specific radioactivity (c/s/μM) |
|-------------------------|---------------------------------|
| Purines:                |                                 |
| Adenine .....           | 110                             |
| Guanine .....           | 110                             |
| Pyrimidine nucleotides: |                                 |
| Cytidylic acid .....    | 180                             |
| Uridylic acid .....     | 180                             |
| Ribose .....            | 0                               |

in the first column, and the appearance of radioactivity in the bacterial component is indicated by a cross, or by a plus and minus where only traces of C<sup>14</sup> appear.

The labeled compounds which the cell has utilized fall into three main classes. In the first class are grouped those compounds which contribute to all four of the bacterial ribonucleotides. Of these, 1-C<sup>14</sup> glucose and C<sup>14</sup>O<sub>2</sub> do not label ribose. In the second class are listed the compounds which label the purines especially, and in the third are those which label the pyrimidines especially.

The interrelationships evident in table 9 are shown in diagrammatic form in figure 18. Thus, carbon atoms 2 to 6 inclusive of glucose have been found to contribute to bacterial ribose. Carbon atom 1 contributes little if at all. The long series of biochemical events taking place between glucose



and ribose (indicated here by means of an arrow) have been intensively studied in other laboratories.

The source of the carbon atoms of the purines was one of the first topics in nucleic acid synthesis to be studied with carbon isotopes. In this very early work, Sonne, Buchanan, and Delluva showed

becomes labeled from  $\text{CO}_2$ , acetate, and glutamate by way of the Krebs cycle.

The results shown here point to the relatively simple materials which can serve as sources of carbon for nucleic acid synthesis in *E. coli*. They also indicate the over-all pattern of the flow of carbon into nucleic acid.

TABLE 9

SOURCES OF NUCLEIC ACID CARBON IN *E. COLI*

All cultures grown in glucose-salts medium with indicated radioactive additive

| Radioactive additive                  | Adenine | Guanine | Cytidylic acid | Uridylic acid | Ribose |
|---------------------------------------|---------|---------|----------------|---------------|--------|
| $\text{C}^{14}$ glucose               | x       | x       | x              | x             | x      |
| 2- $\text{C}^{14}$ glucose            | x       | x       | x              | x             | x      |
| 1- $\text{C}^{14}$ glucose            | x       | x       | x              | x             | ..     |
| $\text{C}^{14}\text{O}_2$             | x       | x       | x              | x             | ..     |
| $\text{HC}^{14}\text{OOH}$            | x       | x       | ..             | ..            | ..     |
| $\text{C}^{14}\text{H}_2\text{O}$     | x       | x       | ..             | ..            | ..     |
| $\text{C}^{14}$ glycine               | x       | x       | ..             | ..            | ..     |
| $\text{C}^{14}$ serine                | x       | x       | $\pm$          | $\pm$         | ..     |
| $\text{C}^{14}$ threonine             | x       | x       | $\pm$          | $\pm$         | ..     |
| $\text{C}^{14}$ aspartate             | $\pm$   | $\pm$   | x              | x             | ..     |
| $\text{C}^{14}$ glutamate             | $\pm$   | $\pm$   | x              | x             | ..     |
| $\text{C}^{14}\text{H}_3\text{COONa}$ | $\pm$   | $\pm$   | x              | x             | ..     |

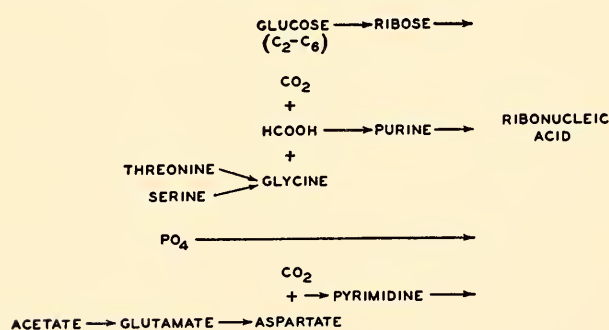


FIG. 18. Sources of ribonucleic acid components

that  $\text{CO}_2$ , formate, and glycine contribute to the purine ring. Similar observations have now been made for a number of organisms, and much is being accomplished toward understanding the details of the way in which the several carbon atoms finally come to rest in the purine ring.

In the case of the pyrimidines,  $\text{CO}_2$ , aspartate, glutamate, and acetate contribute carbon. Aspartate here appears to be the compound of central importance, since it

Not only are studies such as these fundamental to an understanding of integrated activities in the cell, but they can be put to specific use in order to reveal the nature of the more complex chemicals which take part in the construction of polynucleotide strands.

Table 10 gives the results of some experiments in which the incorporation of radioactive carbon dioxide was again studied, but in which the glucose-and-salts medium was supplemented with nitrogenous bases. The results are given in terms of specific radioactivity, which has been expressed as percentage of the value found for the purines in culture 1, the control culture. That is, bacterial adenine of culture 1 has been assigned a value of 100. As we observed earlier, radioactive carbon dioxide labels the purines and the pyrimidine compounds. When the cultures containing supplements are examined, it

is evident that marked suppressions in the incorporation of  $C^{14}$  occur. These suppressions are specifically associated with the kind of supplement used. Thus, in culture 2, in which adenine was added, radioactive carbon dioxide supplies a very small amount of tracer (and hence of carbon) to the nucleic acid purines. The nonradioactive carbon which takes its place in the purines of culture 2 was of course supplied by adenine. Thus the adenine derived from the culture medium is preferentially utilized by the cell to make nucleic acid.

have called it "isotopic competition," since two materials (such as radioactive carbon dioxide and nonradioactive adenine) may be observed to "compete," as it were, for a place in the protoplasm of the intact cell. Its special virtue in the present connection is that it provides a way of probing the inner workings of the cell without the necessity for preparing large numbers of labeled compounds.

Table 11 shows another series of results from competition experiments in which nucleosides and nucleotides have been

TABLE 10  
UTILIZATION OF  $C^{14}O_2$  IN THE PRESENCE OF  $C^{12}$  COMPETITORS

| CULTURE | SUPPLEMENT     | RELATIVE SPECIFIC RADIOACTIVITY OF BACTERIAL COMPONENT<br>(PER CENT OF CONTROL PURINE) |         |                |               |
|---------|----------------|--|---------|----------------|---------------|
|         |                | Adenine  | Guanine | Cytidylic acid | Uridylic acid |
| 1.....  | None (control) | 100  | 100     | 170            | 170           |
| 2.....  | Adenine        | <5   | <5      | 170            | 170           |
| 3.....  | Guanine        | 100  | <5      | 170            | 170           |
| 4.....  | Cytosine       | 100  | 100     | 30             | 30            |
| 5.....  | Uracil         | 100  | 100     | 20             | 20            |

Exogenous adenine is equally effective in supplying carbon to both of the nucleic acid purines; thus we may conclude that adenine can be converted to guanine. Pyrimidine labeling remains unaffected as a result of adenine supplementation. In culture 3, where exogenous guanine was supplied, only polynucleotide guanine could be shown to derive carbon from the exogenous purine. Thus, guanine is not readily, at least, converted to adenine. In cultures 4 and 5, exogenous cytosine and uracil supplied carbon to nucleic acid pyrimidines and were quite freely interconverted. It is evident from these data that external sources of purines and pyrimidines can serve as precursors to nucleic acid. They do so effectively, and specifically, acting as preferred sources of carbon.

The method which is illustrated by these experiments is a perfectly general one. It applies to all sorts of combinations of labeled and unlabeled compounds. We

studied. In the case of the "adenine series" it is evident from the suppressions of  $C^{14}$  utilization that the free base and the riboside are effective carbon sources, but that the nucleotides are relatively poor sources. (The yeast adenylic acid which has been studied here is a mixture of the *a* and *b* isomers.) That they are not so readily utilized as the base or the riboside thus demonstrates that they are not readily converted to these substances.

In the case of the "uracil series" the exogenous materials are about equally effectively utilized to supply the pyrimidine requirements of nucleic acid. Results similar to these are obtained when corresponding "guanine" and "cytosine" series are also studied with radioactive carbon dioxide. In the guanine case, however, there is little conversion to adenine.

These competition experiments with radioactive carbon dioxide show that carbon of the several supplements is preferentially



utilized in nucleic acid synthesis. But they do not reveal the mechanisms by which the supplement is utilized. We do not learn from them whether the ring structure of a base, or the ribosidic linkage in the nucleoside, or the phosphate ester linkage in the nucleotide is preserved during the utilization of the supplement. Each of these problems, however, can be solved by appropriate isotope experiments.

For example, if  $C^{14}$  glucose, uniformly labeled, is supplied to growing cells, and is the only source of carbon available, then

idine nucleotides were carried out. The pyrimidine nucleotides were chosen because as a result of their chemical stability they are relatively easy to prepare.

In these experiments uniformly  $C^{14}$ -labeled cytidylic and uridylic acids were supplied to separate cultures of growing *E. coli*, as were the corresponding  $P^{32}$ -labeled compounds. After an hour's growth the cells of each culture were harvested and analyzed. In addition, the bacterial pyrimidine nucleotides were degraded to yield free cytosine and uracil,

TABLE 11  
UTILIZATION OF  $C^{14}O_2$  IN THE PRESENCE OF  $C^{12}$  COMPETITORS

| CULTURE | SUPPLEMENT            | RELATIVE SPECIFIC RADIOACTIVITY OF BACTERIAL COMPONENT<br>(PER CENT OF CONTROL PURINE) |         |                |               |
|---------|-----------------------|--|---------|----------------|---------------|
|         |                       | Adenine  | Guanine | Cytidylic acid | Uridylic acid |
| 1.....  | None (control)        | 100  | 100     | 170            | 170           |
| 2.....  | Adenine               | <5   | <5      | 170            | 170           |
| 3.....  | Adenosine             | <5   | <5      | 170            | 170           |
| 4.....  | Adenosine-5-phosphate | 50   | 50      | 170            | 170           |
| 5.....  | Yeast adenylic acid   | 50   | 50      | 170            | 170           |
| 6.....  | Uracil                | 100  | 100     | 20             | 20            |
| 7.....  | Uridine               | 100  | 100     | 10             | 10            |
| 8.....  | Uridylic acid         | 100  | 100     | 20             | 20            |

all the carbon locations in the nucleic acid newly made by the cell become labeled. If a similar culture is prepared, but with nonradioactive adenine as a competitor, it is found that polynucleotide purine derives over 90 per cent of its carbon from the exogenous source. Thus, every carbon location of the bacterial purine ring derives some, and very likely all, of its carbon from the exogenous purine. Similar results follow for the corresponding cases of guanine, cytosine, and uracil supplementation. It is reasonable to conclude, therefore, that all the ring structure of the externally supplied nitrogenous base is utilized during the formation of nucleic acid. In order to determine whether the nucleosides and nucleotides are utilized *as such* to form nucleic acid, some experiments with  $C^{14}$ - and  $P^{32}$ -labeled pyrim-

and each of these residues was then analyzed. The results are shown in table 12.

Thus, it was found that in the cultures which were supplied  $C^{14}$  cytidylic acid, an appreciable amount of  $C^{14}$  had been incorporated into the bacterial pyrimidine nucleotides, but that in the parallel phosphorus-labeled culture, practically no radio-phosphorus had been taken up. A similar result was observed for the "uridylic acid" pair of cultures. It has been shown by separate measurements that the cells are permeable to each of the four labeled compounds. It is clear, therefore, that the exogenous pyrimidine nucleotides are incorporated into nucleic acids in a manner which dissociates the phosphorus atom from the carbon atoms. This result has been confirmed by isotopic competition experiments with  $P^{32}$ -labeled inorganic

phosphate and nonradioactive pyrimidine nucleotides. It was again observed that the exogenous nucleotides do not supply phosphorus for nucleic acid synthesis.

Thus externally supplied pyrimidine mononucleotides are utilized to supply carbon, but they are not utilized as intact units for ribonucleic acid synthesis in *E. coli*.

When the distributions of  $C^{14}$  in the bacterial components are determined (table 12) it is observed in each case that the pyrimidine ring and the ribose moiety are about equally labeled. These findings

as intermediates in nucleic acid synthesis, entering as units into the structure of bacterial polynucleotides.

The problem of purine nucleotide utilization was examined by the isotopic competition method, using either  $P^{32}$ -labeled inorganic phosphate or uniformly  $C^{14}$ -labeled glucose as the tracer compound. Neither adenosine-5-phosphate nor the mixture of isomers, yeast adenylic acid, influenced the utilization of labeled inorganic phosphate for the synthesis of nucleic acid. Guanylic acid behaved in a similar fashion, as did inorganic pyrophosphate,

TABLE 12

UTILIZATION OF LABELED PYRIMIDINE NUCLEOTIDES FOR NUCLEIC ACID SYNTHESIS

| LABELED COMPOUND             | SPECIFIC RADIOACTIVITY (c/s/ $\mu$ M) | SPECIFIC RADIOACTIVITY OF BACTERIAL COMPONENT (c/s/ $\mu$ M) |            |          |               |          |          |
|------------------------------|---------------------------------------|--|------------|----------|---------------|----------|----------|
|                              |                                       | Cytidylic acid   | Cytosine * | Ribose † | Uridylic acid | Uracil * | Ribose † |
| $C^{14}$ cytidylic acid..... | 160                                   | 113  | 56.5       | 56.5     | 90            | 49       | 41       |
| $P^{32}$ cytidylic acid..... | 94                                    | ~1   | ...        | ...      | ~1            | ...      | ...      |
| $C^{14}$ uridylic acid.....  | 117                                   | 60   | 33         | 27       | 70            | 36.5     | 33.5     |
| $P^{32}$ uridylic acid.....  | 169                                   | ~1   | ...        | ...      | ~1            | ...      | ...      |

\* From 6 N HCl hydrolyzate.

† Estimated by difference.

imply that *pyrimidine nucleosides* can enter as units into the structure of polynucleotide strands, and indicate that pyrimidine interconversions can occur at the nucleoside level.

These results have been confirmed by a series of competition experiments in which the utilization of  $C^{14}$  cytidylic acid in the presence of nonradioactive cytosine and sugars was studied. Some of the results are given in table 13. These data show that no suppression in the utilization of the labeled material occurred as a result of the presence of cytosine or of the sugar compounds. In addition,  $C^{14}$  ribose is not utilized by these cells. Thus, the nucleotide supplied was utilized in preference to the base or sugar. Since we know that the phosphorus of the pyrimidine nucleotide does not accompany its carbon, it is concluded that pyrimidine nucleosides can act

TABLE 13

SPECIFIC UTILIZATION OF  $C^{14}$  CYTIDYLIC ACID FOR NUCLEIC ACID SYNTHESIS

| SUPPLEMENT *                       | SPECIFIC RADIOACTIVITY OF BACTERIAL COMPONENT (c/s/ $\mu$ M) |               |
|------------------------------------|--|---------------|
|                                    | Cytidylic acid   | Uridylic acid |
| None (control) .....               | 33.0   | 25            |
| Cytosine, ribose .....             | 31.7   | 25            |
| Cytosine, ribose-5-phosphate ..... | 32.5   | 25            |

\* 1  $\mu$ M of  $C^{14}$  cytidylic acid, 2  $\mu$ M of each supplement per culture.

† Adenine and guanine are very weakly labeled, specific radioactivity  $<1$  c/s/ $\mu$ M.

adenosine triphosphate, and a number of phosphorylated sugar compounds. When the various adenylic and guanylic acids were tested with  $C^{14}$  glucose, however,



some utilization could be detected. It appeared, therefore, that exogenous purine nucleotides, like pyrimidine nucleotides, did not enter as units into nucleic acid.

Therefore, purine nucleosides were examined by the isotopic competition method, using  $C^{14}$  glucose as the tracer material. The purine nucleosides were effective competitors, suppressing the uptake of  $C^{14}$  into bacterial nucleic acid. Though they contributed to the purine base, however, they did not appear to contribute to the ribose moiety of nucleic acid purine ribotide. Consequently, a search

of  $C^{14}$  than do those of the control culture. Clearly, carbon from each of the various supplements is being utilized for purine nucleotide synthesis. Examination of the specific effect on the bases and on the ribose reveals, however, that the purine and pyrimidine nucleoside supplements are being used differently. Adenosine contributes largely to the adenine and guanine portions of the nucleotides, whereas cytidine and uridine contribute largely to the sugar moiety. Thus, the ribose portion of the exogenous pyrimidine nucleoside becomes a part of the purine nucleotide seg-

TABLE 14  
UTILIZATION OF  $C^{14}$  GLUCOSE FOR NUCLEIC ACID SYNTHESIS

| SUPPLEMENT           | SPECIFIC RADIOACTIVITY OF BACTERIAL COMPONENT (c/s/ $\mu$ M) |          |           |          |                 |          |           |          |
|----------------------|--|----------|-----------|----------|-----------------|----------|-----------|----------|
|                      | Adenylic acid *  |          | Adenine † | Ribose ‡ | Guanylic acid * |          | Guanine † | Ribose ‡ |
|                      | <i>a</i>   | <i>b</i> |           |          | <i>a</i>        | <i>b</i> |           |          |
| None (control) ..... | 37   | 35       | 20        | 16       | 34              | ..       | 20        | 14       |
| Adenosine .....      | 17   | 17       | 2         | 15       | 17              | 15       | 5         | 11       |
| Cytidine .....       | 23   | 25       | 17        | 7        | 18              | 19       | 13        | 5.5      |
| Uridine .....        | 29   | 28       | 20        | 8.5      | 23              | 25       | 20        | 4        |

\* Specific activity of isomers *a*, *b* respectively.

† After 1 N HCl hydrolysis.

‡ Estimated by difference.

was made for possible sources of the ribose that was attached to the purine ring in bacterial nucleic acid.

Preliminary experiments indicated that pyrimidine nucleosides and nucleotides at moderately high levels might serve as sources of purine ribose. This suggestion was then critically examined with  $C^{14}$  glucose and pyrimidine-containing materials. Table 14 gives some results of competition experiments using adenosine, cytidine, and uridine as competitors in a culture labeled with  $C^{14}$  glucose. In these experiments the routine procedure was modified in order to make available the bacterial purine nucleotides. These compounds were then purified by chromatography and analyzed.

The purine nucleotides of the supplemented cultures contain smaller amounts

of nucleic acid. Under the conditions of this experiment, well over half of the "purine ribose" arose in this way. The details of the mechanisms by which this comes about are not yet known, although in over-all appearance the result is equivalent to what the enzyme chemist recognizes as "transglycosidation." In the example we have been considering, a unit containing a pyrimidine nucleoside would serve as the "ribosyl" donor, and a purine-containing unit, synthesized by the cell from glucose, would serve as the acceptor.

By means of isotopic tracer methods which take advantage of the nutritional characteristics of growing bacteria, it has thus been possible to determine which substances serve as sources of nucleic acid carbon. It has also been possible to insert

suspected precursors at various places along the synthetic streams which form nucleic acid, and to decide what roles they play while contributing to the flow of carbon.

Thus, studies using media supplemented by pyrimidine nucleosides have made it possible to specify some of the processes involved in the synthesis of nucleic acid. In these cases the purines are manufactured from glucose carbon, and the ribose associated with them is derived from the externally supplied pyrimidine nucleoside. This transfer of ribose involves a process much like, and perhaps identical with, transglycosidation. Meanwhile the pyrimidine nucleosides are also incorporated into

nucleic acid as units. The same mechanisms operate with pyrimidine nucleotides. Here, however, the phosphorus of the nucleotide is replaced by phosphorus derived from the inorganic phosphorus of the medium.

Although the exact nature of the mechanism which inserts phosphorus into nucleic acid has not been revealed, we would suggest that phosphorus compounds, perhaps of the uridine-tri-phosphate type, react with end groups to form nucleic acid linkages.

Thus by appropriate choice of culture conditions, reaction sequences involved in the synthesis of the macromolecule can be demonstrated.

## OPERATIONS AND STAFF

### CO-OPERATIVE WORK OF THE DEPARTMENT

Co-operative work has been continued with various governmental, educational, and commercial organizations. These include the Department of Defense, National Research Council, National Institutes of Health, National Bureau of Standards, National Science Foundation, Atomic Energy Commission, Geological Survey, Geophysical Institute of Huancayo (Peru), Catholic and Tulane Universities, University of Pennsylvania, Mount Wilson-Palomar and McMath-Hulbert Observatories, and Cavendish Laboratory (England). We have maintained collaboration with the International Scientific Radio Union and the International Union of Geodesy and Geophysics. Investigators have visited us, some on Carnegie fellowship appointments, from Australia, England, Norway, and Pakistan.

We have continued to work with the Geophysical Laboratory on the age of igneous intrusives. The University of Western Australia has been assisted in assembling equipment for determining the ages of rocks in Australia using isotope and mass-spectrograph procedures. The Department of the Navy has continued to help us with our seismic program.

The observatories at Huancayo, Peru; Christchurch, New Zealand; Godhavn, Greenland; Cheltenham, Maryland; and Climax, Colorado have generously continued their assistance with our cosmic-ray program.

Government contracts, without subsidy, have been continued for investigations of the earth's crust, cosmic rays, and the measurement of the ages of igneous intrusives and other minerals, particularly in Pre-Cambrian rocks.

One staff member is serving on the ad hoc committee to review the program of the Army in combat developments and as a member of the Advisory Panel on General Sciences of the Department of Defense. One staff member continued full time during this report year his research work for the government, and others have acted as consultants for short periods. Another staff member was elected to the National Academy of Sciences; the same man spent several months at the Cavendish Laboratory (England) as a visiting research worker.

The National Science Foundation, the California Institute of Technology, and the Carnegie Institution of Washington sponsored a Radio Astronomy Conference at



the Institution, January 4-6, 1954, attended by some eighty-five astronomers, physicists, and electronic engineers from this country and abroad. An exhibit was prepared for this conference and later shown at the meetings of the National Academy of Sciences and the American Geophysical Union.

#### ADMINISTRATION AND OPERATION

The *Journal of Geophysical Research* has been continued, with subsidy from the Institution to meet part of the publication costs.

Parts of various farms were leased for the researches on radio astronomy and the ionosphere.

#### LECTURES

The following lectures were given by visitors:

November 5, 1953, "Extinct natural radioactivity," T. B. Kohman.

December 3, 1953, "Studies in ion exchange equilibria," H. F. Walton.

January 7, 1954, "The structure of nucleic acid," F. H. C. Oxley.

January 20, 1954, "The double scattering of protons," C. L. Oxley.

February 10, 1954, "Random processes," A. F. J. Siegert.

May 13, 1954, "Free nuclear precession," E. L. Hahn.

June 21, 1954, "The action of penicillin on protein synthesis," E. F. Gale.

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WYCKOFF, J. M. See TEMMER, G. M.

## STAFF AND ORGANIZATION

### SCIENTIFIC STAFF

*Director:* M. A. Tuve.

#### *Staff Members*

*Geophysics:* L. T. Aldrich, B. F. Burke, J. W. Firor, S. E. Forbush, J. W. Graham, E. A. Johnson,\* H. E. Tatel, G. R. Tilton, E. H. Vestine, H. W. Wells, G. W. Wetherill.

*Laboratory and Biophysics:* P. H. Abelson,† E. T. Bolton, R. J. Britten, D. B. Cowie, N. P. Heydenburg, R. B. Roberts, G. M. Temmer.

*Guests, Associates, Fellows, and Visiting Investigators:* S. J. Ahmed, Pakistan; S. Chapman, England; Mrs. J. K. Doherty, National Institutes of Health; W. R. Duryee, National Institutes of Health; G. Field, Princeton University; L. B. Flexner and Mrs. J. B. Flexner, University of Pennsylvania; P. W. Gast, Lamont Geological Observatory; P. J. Hart, Harvard University; H. L. Helfer, Yerkes Observatory; J. Z. Klose, Naval Academy; R. D. McAfee, Tulane University; B. Y. Mills, Radio Physics Laboratory, Sydney, Australia; Leif Owren, Institute of Theoretical Astrophysics, Oslo, Norway; Mrs. I. Z. Roberts; F. G. Smith, Cavendish Laboratory, Cambridge, England;

\* On leave of absence for government work.

† Director of Geophysical Laboratory from September 1, 1953.

M. Sugiura, University of Alaska; F. L. Talbott, Catholic University; R. E. Trumble, Jr., Catholic University; H. F. Walton, University of Colorado.

### OPERATING STAFF

*Administrative:* M. B. Smith, W. F. Steiner.

*Office and Clerical:* Mrs. C. C. Ator, W. N. Dove, W. C. Hendrix, Mrs. A. P. Moffett, D. J. O'Rourke, Miss H. E. Russell, Mrs. F. B. Silberstein.‡

*Instrument Shop:* B. J. Haase, L. A. Horton, J. G. Lorz.

*Research Assistants and Laboratory Assistants:* S. J. Buynitzky, H. E. Cronin, J. B. Doak, E. T. Ecklund, Miss E. F. French, R. E. Hewitt, P. A. Johnson, C. J. Ksanda, Mrs. J. G. Lappin,‡ C. A. Little, Jr., Mrs. E. A. MacKenzie,‡ M. H. MacKenzie,‡ Miss B. K. Phillips, R. W. Reuschlein, W. E. Scott.

*Computer:* Miss I. Lange.

*Maintenance:* C. Balsam, C. R. Domton, C. P. Giffin,‡ F. C. McGrady, E. Quade, S. Swankowski.

*Part-time and Temporary Employees:* Fifteen part-time and temporary employees were engaged during the year, usually for short periods, to assist in the office and laboratory work.

‡ Resigned.

## GEOPHYSICAL LABORATORY

*Washington, District of Columbia*

PHILIP H. ABELSON, *Director*

With the advent of a new Director, an evaluation of the role of the Geophysical Laboratory is in order. Questions requiring consideration are the broad charter, the past and present relation of the Geophysical Laboratory to science and technology, the policy with respect to personnel, and a research program for the present and immediate future.

The Geophysical Laboratory was created following a report by a distinguished committee which envisioned grand objectives. It recommended that a central laboratory of geophysics be established at Washington "to study in a comprehensive way the great area in which geology, physics and chemistry intermingle." The broad field to which attention was directed at that time included "(1) *the great envelopes of the earth*, the atmosphere and the hydrosphere, which constitute the chief sources of external activities and condition the habitability of the globe; (2) *the body of the earth*, whose crust records its history, and whose interior is full of dark, intricate problems. . . ." It was urged that special experiments and techniques be devised to cope with the problems involved, and that the results of experiment be applied "with the most critical circumspection to the phenomena which the earth itself presents"; and it was stated that "the soul of the method should be to determine by the grandest and at the same time the most refined artificial experiments what is the meaning of the magnificent experiments expressed in the evolution of the earth."

Interpreted broadly, this charter is timeless. If its mandates are executed with vigor and imagination and the opportunities of each epoch are met, it can serve as a guide for generations to come. It is as inspiring today as it was fifty years ago. With passage of the years many questions

have been examined, new puzzles have arisen, and new techniques have evolved that have made additional areas of study attractive and accessible. The basic challenges remain.

In many ways the Geophysical Laboratory occupies a unique position in a field where areas of science and technology overlap. It has made major contributions in both pure and applied research. It has contributed to physical chemistry and geology. It has served as a leading interpreter and advocate of application of the exact sciences, including physics and chemistry, to solution of problems found in the earth sciences. Examination of five of the principal advanced textbooks on petrology reveals that 25 per cent of the literature cited covers work that was performed at the Geophysical Laboratory. The Laboratory has also made important contributions through its work in mineralogy and volcanology. Establishment of the scale for high temperatures, phase equilibrium studies, and other physical-chemical investigations have given the Laboratory a place in the physical sciences. Today the Laboratory is one of the leading investigators of chemical phenomena at very high pressures.

Technology has made important applications of the experimental results obtained at the Geophysical Laboratory. The basis for the modern manufacturing procedures in the two-billion-dollar Portland cement industry is the paper by Rankin and Wright entitled "The ternary system  $\text{CaO}-\text{Al}_2\text{O}_3-\text{SiO}_2$ ." This study of the melting behavior of mixtures of lime, alumina, and silica has been called the bible of the Portland cement industry, because it has solved scientifically the age-old riddle of cement and has stood for subsequent years as a sound guide for the



industry. Another scientific study of basic industrial importance is "The system  $\text{Al}_2\text{O}_3\text{—SiO}_2$ " by Bowen and Greig, which showed that the place to look for improved refractories was not in the 1:1 molecular ratio of the two oxides, as had been supposed, but in the 3:2 ratio of the new mineral mullite. Another technological application of our researches is in glass manufacture. Man's use of glass dates back thousands of years, and from the earliest times until recently all glass consisted essentially of soda, lime, and silica, with certain adventitious, though important, impurities. Publication of "The ternary system sodium metasilicate—calcium metasilicate—silica" by Morey and Bowen showed for the first time which of the ternary melts could be readily quenched to glass.

The absence of a broad research policy on the part of the mineral and fuel industries has enhanced the importance of the role of agencies devoted to basic research. The resources of the earth are crucial to man's economy and way of life. In the past it has been possible to exploit the fairly obvious resources, such as the rich ore bodies outcropping on the surface. In the future the exploitation of natural resources will be more difficult, and it will be necessary to understand far better the structure and evolution of the earth to find its hidden wealth. The mineral industries have devoted their energies to short-term exploitation rather than to the accumulation of that wealth of understanding which gives the key to problems yet unsuspected and unseen. In so far as basic questions have been asked, they have been posed by geologists. But geology uses the principles and facts of physics, chemistry, and biology. The forefront of much that is new and quantitative in the earth sciences is therefore found in the laboratory discoveries which are directed toward understanding the origin and evolution of the earth.

Effective investigators in this field must possess training and judgment in both physical chemistry and geology. This com-

bination is rare. A limited number of geologists have managed a fair mastery of physical chemistry. Few physical chemists have undertaken work in geology. As a result there are today perhaps a few score laboratory investigators supplying new quantitative criteria for the interpretation of field problems encountered by the ten thousand geologists of this country.

Research in such a borderline field presents unique intellectual opportunities for a laboratory and its staff. Very few men have adequate formal training in more than one discipline. At the Geophysical Laboratory is a group of men having a common bond of interest in earth science, but with divergent backgrounds in physics, chemistry, biology, and geology. These men stimulate and assist one another to learn more about the fields in which their formal training is deficient. Under such circumstances men continue to grow long after their graduate days are past, and they achieve the flexibility necessary to meet the changing challenges of research. Continued intellectual growth of staff members is fully as important as output of research reports.

For many years the main program of this Laboratory involved investigations of the formation of the igneous rocks through phase equilibrium studies involving eight major oxides— $\text{SiO}_2$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{FeO}$ ,  $\text{Fe}_2\text{O}_3$ ,  $\text{Na}_2\text{O}$ ,  $\text{K}_2\text{O}$ ,  $\text{CaO}$ , and  $\text{MgO}$ . Our work on anhydrous silicate equilibria continues, but techniques developed shortly after World War II have permitted rapid expansion of studies involving  $\text{H}_2\text{O}$  as a component, and these are for the first time providing a firm experimental basis for the interpretation of the metamorphic rocks. This work involves the study of formation of minerals under pressures as high as 200,000 pounds per square inch, with temperatures from  $25^\circ$  to  $1500^\circ\text{C}$  in the presence of controlled pressures of  $\text{H}_2\text{O}$ . This work is currently making striking progress and is yielding results which are applicable to vast portions of the crust throughout the world. Indeed, it is this universal aspect

of much of the work of the Geophysical Laboratory that has helped win for it such an important role in the earth sciences. These hydrothermal investigations will be prosecuted vigorously and will continue to form a major portion of our program.

Another investigation which will be continued is the study of solubility of minerals in fluids such as  $H_2O$  at elevated temperatures and pressures. In addition to pioneering a new realm of physical chemistry, these studies provide a basis for understanding the mechanisms of transport of huge quantities of minerals in geological processes. Another continuing series of studies, recently initiated, is the application of modern small-sample statistical methods to descriptive petrography. This work has been concerned chiefly with inquiry into the distribution of major minerals and elements in common rocks, and development of rapid methods of determining composition.

The Laboratory will be alert to new opportunities to spearhead research in other areas pertinent to our broad charter. Scouting ventures by individual staff members will serve to determine the feasibility of new research programs. Two such

explorations were made this year, and both have uncovered areas suitable for further work. One of these has been an inquiry into the behavior of trace elements in geological processes. The distribution of trace elements between fluids and newly formed crystals is being measured at temperatures and pressures similar to those at which igneous rocks were formed. This study will yield new information regarding the processes of formation of crystals and will provide a sound quantitative basis for interpreting distributions of trace elements in mineral assemblages.

Another new area of research initiated this year at the Laboratory is the study of organic constituents of fossils. These investigations include identification of the compounds and measurement of their stability as a function of time and temperature. Examination of the organic constituents of fossils may open new vistas of knowledge concerning creatures living in the past. It may also provide a new kind of recording thermometer for sediments. This and other researches carried out during the past year are described in more detail in the following sections of this report.

## PALEOBIOCHEMISTRY

*Organic constituents of fossils* (Abelson). Organic material equivalent in quantity to the weight of the earth has been created by living creatures since life originated on this planet. Almost all of this has been metabolized by other creatures, with a few notable exceptions. Under special conditions of deposition and burial, the organic matter represented by coal, oil, and oil shales has been preserved. Some of the chemicals found in these fuels are only moderately altered products of compounds originally part of living organisms.

Examination of a variety of fossil shells and bones has now revealed that small but important quantities of organic materials are often preserved within these fossils. Some of the amino acid building blocks—

alanine, glutamic acid, and valine—which are important constituents of today's proteins have been found in fossils as old as 360 million years.

Isolation of the organic compounds from fossils was accomplished through use of a combination of ion-exchange and paper-chromatography techniques. The fossils were first dissolved in acids, and any proteins present hydrolyzed. The solution was freed of mineral constituents by passage through a resin column. After elution from the resin, the mixture of amino acids was resolved on a paper chromatogram. Positive identification of the individual compounds was achieved by addition of radioactive tracers to some of the unknown mixtures.

Recent shells and calcareous materials



of biological origin have been examined as a guide to the understanding of occurrences of organic materials in fossils. The presence of proteins in bones has long been established, but virtually nothing was known about the nature of organic constituents in shells. In table 1 are shown results of examination of some tropical shells. It will be noted that all specimens listed contained amino acids. Twenty other recent, arbitrarily chosen shells were surveyed. All contained amino acids in

attack. A further requirement is that the inorganic parts of the fossil should not be dissolved or replaced by other substances. A third requirement is that the organic material should not diffuse from, or be in any way transported away from, the inorganic matrix. Finally, the organic compounds must have sufficient chemical stability to exist unchanged for long periods of time.

The distribution of proteins within typical shells was investigated. By exposure

TABLE 1  
AMINO ACID CONTENT OF VARIOUS RECENT TROPICAL CALCIUM CARBONATE SHELLS

| Specimen                        | Description         | Mineral composition                              | Amino acid content * |
|---------------------------------|---------------------|--|----------------------|
| <i>Porites lichen</i> .....     | Reef-building coral | Aragonite, 10-20 per cent calcite                | 6.7                  |
| <i>Porolithon onkodes</i> ..... | Coralline alga      | Calcite with MgCO <sub>3</sub> in solid solution | 17.6                 |
| <i>Chama lazarus</i> .....      | Reef-dwelling clam  | Aragonite  | 16.4                 |
| <i>Codakia punctata</i> .....   | Ribbed clam         | Aragonite  | 8.4                  |
| <i>Atactodea glabrata</i> ..... | Intertidal clam     | Aragonite  | 42.7                 |
| <i>Cymatium muricinum</i> ..... | Thick-shelled snail | Aragonite  | 20                   |
| <i>Tridacna maxima</i> .....    | Man-eating clam     | Aragonite  | 26.6                 |
| <i>Cypraea mauritiana</i> ..... | Tropical snail      | Aragonite  | 6.2                  |

The materials were furnished by Preston Cloud, U. S. Geological Survey. The *Porites lichen* and *Porolithon onkodes* were collected at Saipan. The other specimens were collected at Onotoa Atoll in the Gilbert Islands.

\* In micromoles per gram ( $\mu\text{M/g}$ ). One  $\mu\text{M/g}$  is approximately equal to 1 part per 10,000.

amounts ranging from 6 to 40 micromoles per gram. The amino acids appearing were those typically found in protein hydrolyzates, with occasional specimens showing additional unidentified components. One may conclude that the occurrence of proteins in recent shells and other hard parts of invertebrates is a general phenomenon.

This survey makes it seem likely that many fossils originally contained proteins. It leads directly to the question of what are the favorable circumstances for preservation of these substances. It seems obvious that the first condition is freedom from bacterial destruction. Organic constituents preserved within a dense inorganic matrix, such as a shell, would be safe from such

to controlled amounts of dilute acid it is possible to remove layers from shells and thus to determine the location of the protein. Results of such a procedure are shown in table 2. It will be noted that, although the protein was not uniformly distributed throughout the shell, it was present in all portions. Many of the shells are dense, solid structures, and their interiors are therefore quite impervious to bacterial action. Inspection of fossils and X-ray determination of their constituent minerals permit investigation of degree of replacement. The problem of diffusion is more difficult to assess, and no good quantitative approach has yet been devised. It seems likely, however, that diffusion is not too important, and that long-time





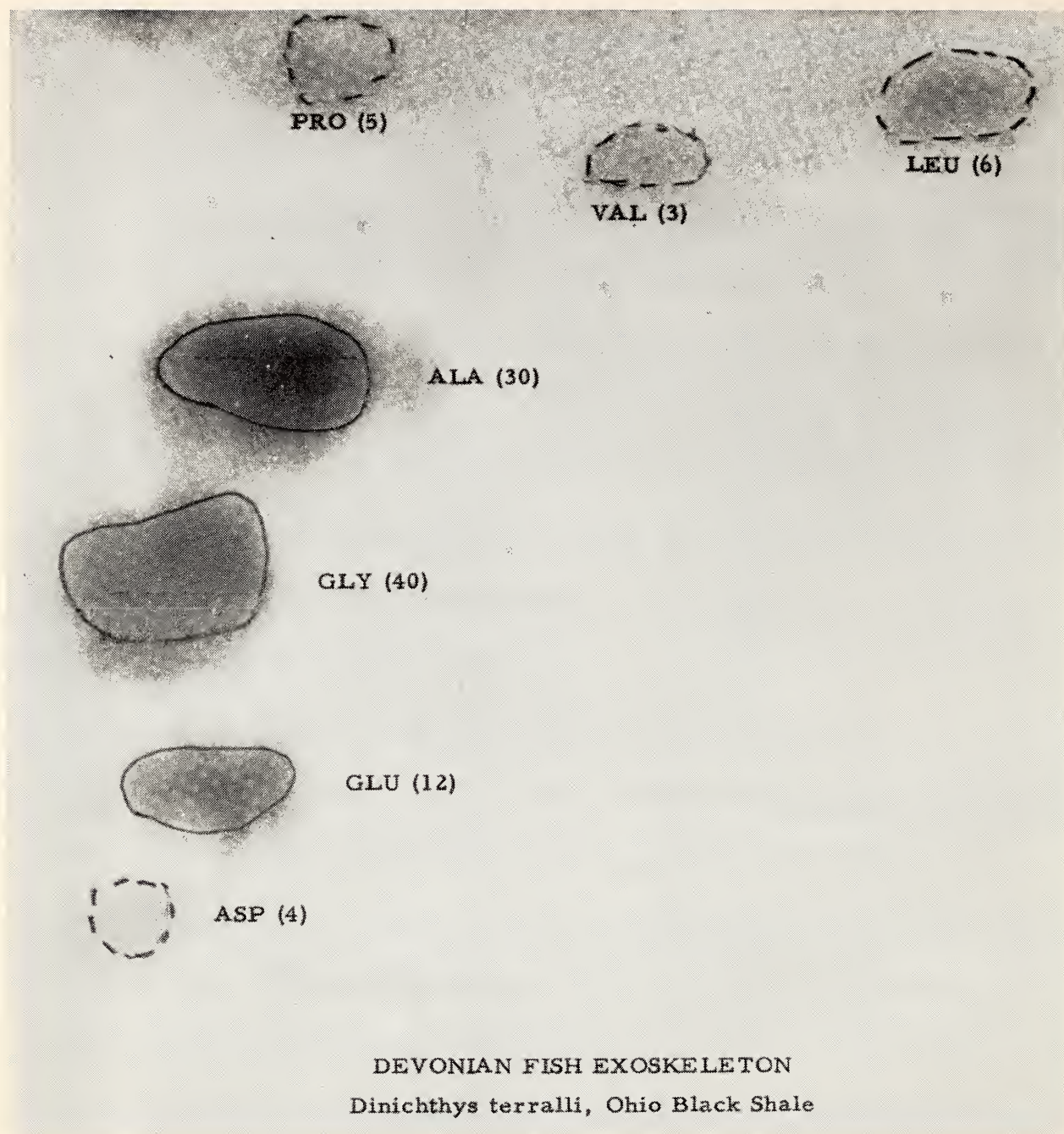


FIG. 1. Two-dimensional paper chromatogram of amino acids separated from a 360-million-year-old fossil. Ala, alanine; asp, aspartic acid; glu, glutamic acid; gly, glycine; leu, leucine; pro, proline; val, valine.

preservation of amino acids is most crucially dependent on the intrinsic thermal stability of the various compounds and on the thermal conditions to which the fossils have been exposed.

With these considerations in mind, a series of fossils showing no silicification or replacement and chosen on the basis of probable mild thermal history were ex-

amined. Both shells and bones were studied. In all cases the fossils contained amino acids. Results from some teeth and bones are given in table 3. These results show no trend with time, which is not too surprising, since different burial histories were involved. Actually the oldest fossil in this group was richest in amino acids. It is possible that this was due in part to the preserving black shale matrix. Black shales are formed in anaerobic environments, often under virtually sterile conditions. A chromatogram of amino acids separated from a Devonian fish plate is shown in figure 1. Amino acids from fossils have been shown to be identical with present-day compounds, by use of radioactive tracers. The amino acids found in the fossils listed in table 3 and shown in figure 1 are those which laboratory tests have shown to be particularly thermally stable. They are precisely those amino acids which could survive if a modern protein were to be stored in a fossil for similar periods of time. It therefore seems likely that these fossils originally contained proteins which played the same important role in the lives

TABLE 2

SERIAL SOLUTION OF A RECENT CLAM SHELL

| Fraction (%)  | Amino acid content ( $\mu\text{M/g}$ ) | Protein ( $\%$ ) |
|---------------|--|------------------|
| 10-20.....    | 11.7                                   | 1.24             |
| 20-30.....    | 12.3                                   | 1.31             |
| 30-40.....    | 17.8                                   | 1.90             |
| 40-50.....    | 20.3                                   | 2.17             |
| 50-60.....    | 22.0                                   | 2.35             |
| 60-70.....    | 17.8                                   | 1.90             |
| 70-80.....    | 15.0                                   | 1.61             |
| 80-90.....    | 10.2                                   | 1.09             |
| Average ..... | 15.9                                   | 1.70             |

The specimen used was *Mercenaria mercenaria*, an edible clam grown at Cape May, New Jersey.

TABLE 3

AMINO ACID CONTENT OF VARIOUS FOSSILS

| Name   | Approximate age (years)          | Formation                     | Amino acid content ( $\mu\text{M/g}$ ) | Principal constituents       |
|--|----------------------------------|-------------------------------|--|------------------------------|
| <i>Plesippus</i> (prehistoric horse).....      | Late Pliocene<br>$5 \times 10^6$ | Hagerman Lake Beds, Idaho     | 0.6                                    | Ala, gly                     |
| <i>Plesippus</i> (prehistoric horse, tooth)... | Late Pliocene<br>$5 \times 10^6$ | Hagerman Lake Beds, Idaho     | 1.5                                    | Gly, ala, leu, val, glu      |
| <i>Mesohippus</i> (prehistoric horse, tooth) . | Oligocene<br>$40 \times 10^6$    | White River, Nebraska         | 0.31                                   | Ala, gly                     |
| <i>Mosasaurus</i> (dinosaur) .....             | Cretaceous<br>$100 \times 10^6$  | Pierre Shale, South Dakota    | 1.8                                    | Ala, gly, glu, leu, val      |
| <i>Anatosaurus</i> (dinosaur) .....            | Cretaceous<br>$100 \times 10^6$  | Lance, Lance Creek, Wyoming   | 2.8                                    | Ala, gly, glu, leu, val, asp |
| <i>Stegosaurus</i> (dinosaur) .....            | Jurassic<br>$150 \times 10^6$    | Morrison, Como Bluff, Wyoming | 0.26                                   | Ala, gly, glu                |
| <i>Dinichthys</i> (fish) .....                 | Devonian<br>$360 \times 10^6$    | Ohio Black Shale              | 3.0                                    | Gly, ala, glu, leu, val, asp |

Specimens were furnished from the U. S. National Museum by C. Lewis Gazin and David H. Dunkel.

Ala, alanine; asp, aspartic acid; glu, glutamic acid; gly, glycine; leu, leucine; val, valine.



of ancient creatures as they do in today's organisms.

One contingency which must be faced is the possibility that the amino acids found may be adventitious contaminants. Tests in which  $\text{CaCO}_3$  was precipitated in the presence of amino acids showed that aspartic and glutamic acids are the two amino acids which are readily adsorbed. Alanine and glycine, the principal amino acid constituents of fossils, have almost no

degradation, and that alanine, glycine, glutamic acid, leucine, and valine are relatively resistant to heat. Alanine was selected for more detailed study. In dilute solution the rate of degradation of alanine is proportional to concentration. This is the condition for a first-order reaction. Studies at temperatures ranging from  $188^\circ$  to  $310^\circ \text{C}$  showed a reaction rate strongly dependent on temperature. Other measurements revealed that the principal reac-

TABLE 4  
AMINO ACID CONTENT OF FOSSILS FROM ONE LOCALITY

| Specimen                               | Description | Mineralogy                     | Amino acid content ( $\mu\text{M/g}$ ) |
|--|-------------|--------------------------------|--|
| <i>Glycymeris parilis</i> . . . . .    | Clam        | Aragonite                      | 0.80                                   |
| <i>Mercenaria mercenaria</i> . . . . . | Clam        | Aragonite                      | 0.75                                   |
| <i>Melosia staminea</i> . . . . .      | Clam        | Aragonite                      | 0.90                                   |
| <i>Lyropecten madisonius</i> . . . . . | Scallop     | Calcite                        | 1.10                                   |
| <i>Eucrassatella melina</i> . . . . .  | Bivalve     | Aragonite                      | 0.60                                   |
| <i>Turritella variabilis</i> . . . . . | Snail       | Aragonite                      | 0.40                                   |
| <i>Ecphora tricostrata</i> . . . . .   | Snail       | Inner aragonite, outer calcite | 1.20                                   |
| <i>Turritella indenta</i> . . . . .    | Snail       | Aragonite                      | 0.50                                   |

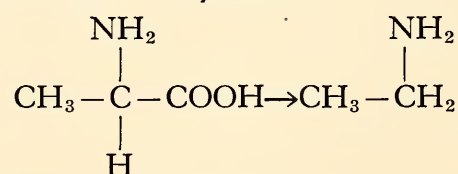
Fossils collected at Plum Point, Zone 10, Calvert Formation, Maryland, by Wendell Woodring, Kenneth Lohmann, and Philip H. Abelson. Age of shells, about 30 million years.

Amino acids present included alanine, glycine, leucine, valine, glutamic acid, and aspartic acid.

affinity for  $\text{CaCO}_3$ , nor is it likely that they have affinity for bone. It seemed desirable, however, to investigate the amino acid content of a group of fossils collected at one locality. These presumably have been exposed to an identical thermal history and have been similarly exposed to percolating ground waters. The shells showed both quantitative and qualitative differences in amino acid content, a fact which minimizes the possible influence of adsorption from ground water. Results are shown in table 4. This problem should be studied further on material from other localities.

In order to begin to interpret the geological significance of the occurrence of amino acids in fossils, it was necessary to survey the thermal stability of these compounds. Experiments showed that there is considerable variation in rates of thermal

tion occurring at these various temperatures was decarboxylation:



With reasonable confidence, therefore, one can apply the experience relative to first-order reactions which has been accumulated by physical chemists.

It has been shown that  $dc/dt = -kc$ ,  $k = \sim 10^{13} e^{-E/RT}$ , where  $c$  is concentration,  $E$  an activation energy,  $R$  the gas constant, and  $T$  the absolute temperature. The value  $10^{13}$  is a frequency factor deduced from a variety of investigations of first-order reactions performed elsewhere. Data obtained in studies of the degradation of alanine are shown in figure 2. The straight

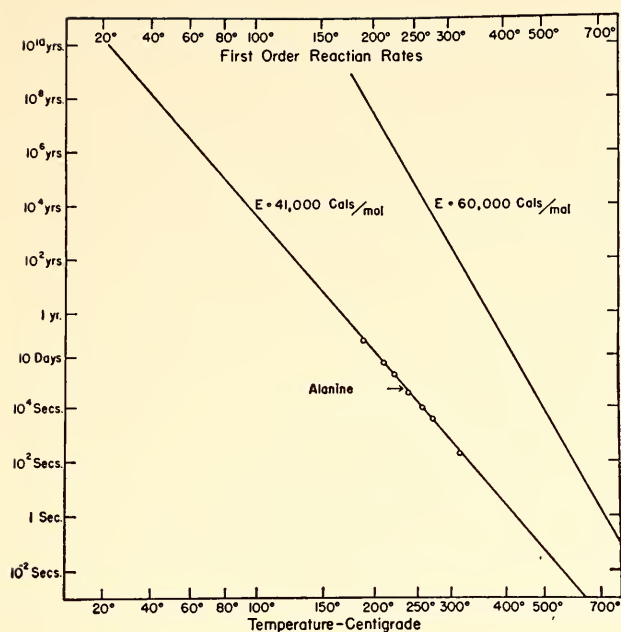


FIG. 2. Thermal degradation of alanine. Time required for decomposition of 63 per cent of original material.

line is based on the equations applicable to a first-order reaction normalized at  $258^{\circ}\text{C}$ . It will be noted that a very large extrapolation is necessary to determine stability at room temperatures. This extrapolation seems justified, however, since the alanine

is actually observed in ancient fossils. It indicates that under mild thermal conditions alanine is sufficiently stable to last more than a billion years. Some of the other organic substances are more stable. Molecules degrading by first-order reactions with an activation energy of 60 kilocalories per mole could last a time equivalent to that since the earth first cooled, even if exposed to temperatures of  $150^{\circ}\text{C}$ .

The thermal studies on alanine suggest the possibility of a geological thermometer for sediments. Although the amount of degradation of a substance depends on both time and temperature, rates of reaction are more strikingly influenced by temperature than by time. Ordinarily time can be estimated from the fossil record. Thus, if amino acids are found in a fossil, an upper limit can be set on the temperature to which the fossil has been exposed. This in turn can be related by means of geothermal gradients to an estimate of burial history. Further development of this method may result in a very valuable tool for the geologist.

## GEOCHEMISTRY OF THE LESS ABUNDANT ELEMENTS

The role of the trace elements in geological processes is an area for research that development of modern techniques has made increasingly accessible to study. Behavior of twenty or thirty elements, ranging in abundance from a few hundredths to a few millionths of one per cent, can now be studied effectively in a great variety of minerals and rocks.

Trace elements have a wide range of ionic properties, and understanding of the distribution of these substances in rocks and minerals may constitute invaluable information for reconstructing geological events. The economic value of many of the trace elements has been appreciated for centuries, and the processes by which they may be concentrated into ore deposits have long been of interest. Geologists and geochemists are now recognizing that many of the trace elements can be helpful, and

often indispensable, in solving many other problems. Of the different areas of application of trace elements in geochemistry, three will be mentioned briefly here.

Any theory of the formation of the chemical elements, the earth's crust, or the earth itself, or any theory of the relations of the earth to the solar system must fit the measured abundance of all the elements. Determination of the abundance of all elements in rocks and minerals of widely different origin and history, as well as in meteorites, is, therefore, an important task of geochemistry.

The distribution of trace elements in rocks and minerals is often very characteristic and revealing to the geologist. Many trace elements follow definite patterns of behavior in a given environment, but these patterns are not reflected in average abundances. Only a detailed under-



standing of the mode of occurrence of trace elements will make this information helpful in solving such problems as determination of the origin of certain rock types, of relations between rocks and between minerals, and of the sequence of geological events. Eventually it may be possible to predict the path that a trace element follows during any well defined rock-forming process.

The presence of trace elements in minerals and rocks can furnish even more specific information. The absolute time scale for the earth's history is based exclusively on radioactive trace elements. The distributions of many other trace elements also can be used as records of the past—records revealing the physicochemical conditions of formation of rocks and minerals, the environment and processes that led to their formation, and the nature of changes and transformations during the succeeding periods. Since many of the variables in the natural processes are quantitatively unknown, these records can be read only with the aid of data procured from controlled experiments with trace elements in synthetic systems. In this field trace elements give promise of very wide application.

From these three areas, two problems have been selected and studied with considerable success: determination of distribution coefficients of trace elements, and investigation of absolute ages.

*Distribution coefficients of trace elements* (Eugster). One of the new researches in which the Laboratory is now engaged is the study of distribution of trace elements in minerals. A wealth of information is stored in the distribution of trace elements between different mineral phases. The principal difficulty in interpretation of studies on natural minerals lies in the number and complexity of the variables. Experimental work on well defined systems, therefore, must be undertaken in conjunction with studies on natural materials.

In the past thirty years a mass of data

has been accumulated on the concentration of certain elements in natural minerals and rocks, mostly with the aid of the optical spectrograph; but only a qualitative guide for inquiry about the behavior of these elements during the course of crystallization or recrystallization has been gained.

The distribution of a trace element between a mineral and the liquid, fluid, or gaseous phase from which it crystallizes can be studied only in special cases, i.e., if the gas phase has been trapped in inclusions or the liquid phase quenched as glass. The distribution of a trace element between two crystalline species that have crystallized in equilibrium with each other is easily accessible to investigation if the grain size of the rock permits clean separation. This distribution factor,  $F$ , given as the ratio of the concentration of the trace element in the two minerals, is probably characteristic of the temperature and pressure of formation of these minerals. A temperature dependence of this factor has been suspected previously, and several indications of it have been cited, but only controlled experiments can furnish a definite and quantitative answer. It is also necessary to determine whether this factor is independent of the total concentration of the trace element, i.e., whether the law of mass action holds for these heterogeneous reactions.

In the case of natural minerals, the distribution factor can be measured, but three of the variables are not determinable—temperature, pressure, and concentration of the trace element in the phase from which the mineral crystallizes. In order to eliminate some of the variables, the equilibrium for a trace element between liquid, or gas, and more than one solid phase must be measured with the aid of synthetic studies. If the temperature influence for two minerals shows enough difference, it should be possible to derive from two sets of data the temperature of formation and original concentration of the trace element in the liquid or gaseous

phase, provided that the two minerals crystallized in equilibrium with each other and that the pressure is constant or of minor importance.

Crystallization under equilibrium conditions can be treated as a heterogeneous chemical equilibrium for which the mass action law applies; therefore

$$cIm + cHx = cIx + cHm$$

and

$$F = \frac{cIx \cdot cHm}{cIm \cdot cHx},$$

where  $Hm$  denotes concentration of the host element in the liquid phase;  $Hx$ , concentration of the host element in the crystal;  $Im$ , concentration of the trace element in the liquid phase;  $Ix$ , concentration of the trace element in the crystal;  $cHm$ , change of the concentration of  $H$  in the liquid phase during the time interval of crystallization;  $cHx$ , change of the concentration of  $H$  in the crystal during the time interval of crystallization;  $cIm$ , change of concentration of  $I$  in the liquid phase during the time interval of crystallization;  $cIx$ , change of concentration of  $I$  in the crystal during the time interval of crystallization; and  $F$ , distribution factor.

If the mass-action rule holds, the distribution factor  $F$  is a constant for a given pressure and temperature. The equation can be rearranged to show the relations better:

$$\frac{cIx}{cHx} = F \frac{cIm}{cHm},$$

where  $F = f(P, T)$ . Of these three entities only the ratio  $Cx = cIx/cHx$  can be measured directly for natural specimens. In an experiment, the composition of the liquid phase  $Cl = cIm/cHm$  can be controlled; and  $F$  as a function of  $(P, T)$  can be determined in a series of experiments for a particular trace element in a particular crystal lattice.

The nature of the liquid or gaseous phase is important for the function  $F = f(P, T)$ . For a simple binary system involving a dry melt,  $F = f(T)$  is not a function independ-

ent of the bulk composition of the system, but for a given temperature and bulk composition a fixed value can be assigned to  $F$ . This value can be taken directly from the positions of the liquidus and solidus curves. For a system involving water in a gas phase,  $F = f(P, T)$  is not dependent on the total concentration of the trace element, and therefore  $F$  has to be determined directly.

Experimental work has been started on the potassium-bearing minerals sanidine and phlogopite to test the behavior of small amounts of the rare alkali metals rubidium and cesium. The minerals are grown in a hydrothermal environment containing known amounts of the rare elements, which are tagged with radioactive isotopes. The trace elements are added to either the liquid phase (water) or the solid synthetic mixture. After each run, the concentrations of the trace elements are determined in the gas phase and in the crystal by radioactive assay. The function  $F = f(T)$  is obtained by varying the temperature. A summary of the results of the experiments on the distribution of cesium between sanidine and the vapor phase in equilibrium with it is presented in figure 3. There is considerable variation of the distribution factor  $F$  with temperature; higher temperatures greatly favor the entrance of cesium into the feldspar lattice.  $F$  is determined as the ratio of the values mg Cs/mg K in sanidine over mg Cs/mg K in the vapor phase, in equilibrium with the crystal.

$F$  is independent of the absolute concentration of cesium for only a fairly narrow range with an upper limit of 0.01 for the ratio mg Cs/mg K in the vapor phase. All experiments have been performed at a water vapor pressure of 1000 bars, and the influence of pressure on  $F$  is not yet known. Similar curves are being determined for rubidium and thallium in phlogopite and sanidine.

These experiments represent the first direct determinations of distribution fac-



tors of minor elements in silicates. It is clear that the same reasoning and the same laws apply for the major elements.

Natural samples of micas and feldspars are being analyzed for their alkali content in an effort to tie the laboratory results in with natural material. The alkali metals are being separated with ion-exchange columns and determined with flame photometer and polarograph.

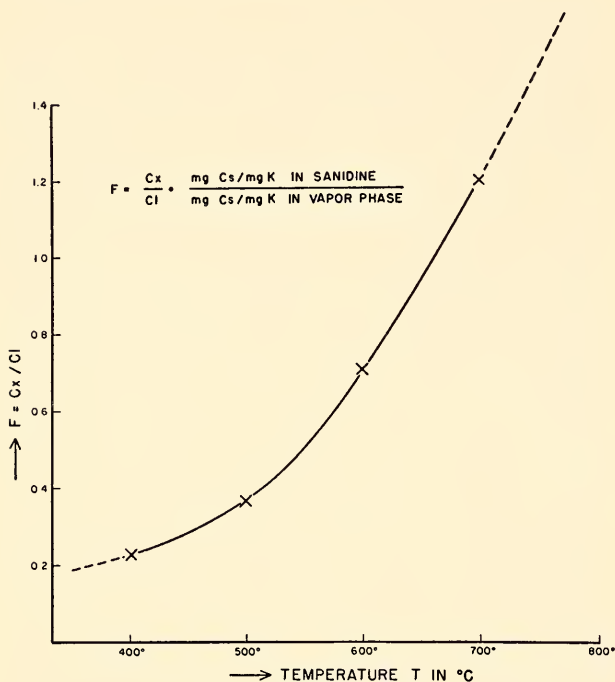


FIG. 3. Variation of the distribution factor  $F$  with temperature for cesium in sanidine at 1000 bars water vapor pressure.

*Age of rocks* (Davis). Any theory of the formation of the crust of the earth and any description of its subsequent history must involve a time schedule of events. The only source of such a calendar is the earth's crust and the rocks of which it is composed. Somewhere in the rocks the calendar-maker must be able to recognize signs which can be translated into numbers. The accuracy with which the translations may be made is important, but still more important is the inherent reliability of the original imprints in the rocks. Have they persisted unchanged since the rock formed? How can we be reasonably sure that they have? Geological evidence may be trusted for young rocks, but during the

vast expanse of Pre-Cambrian time many complicated events may have occurred. The best that can be done is to take specimens that appear to have survived unchanged and study these to see if all the signs point to a single age.

Three years ago, a co-operative program between the Department of Terrestrial Magnetism and the Geophysical Laboratory was initiated to study the age problem, with the specific aim of comparing results obtained from the several radioactive decay schemes, which are the self-contained calendars of the rocks. Granite and pegmatites have been selected because of their widespread occurrence in the crust, because they often appear to be unaltered, and because they are composed in minor part of minerals such as micas, zircon, and sphene. These minerals contain somewhat more of the radioactive elements rubidium, uranium, and thorium than does the rest of the rock. Potassium is also present in the granite. Each of these elements has partially decayed in the rock and has formed small amounts of new elements. Since the rates of decay are known and the ratio of parent to daughter element is measured, an age can be determined. The potentially usable parent-daughter pairs are  $U^{238}/Pb^{206}$ ,  $U^{235}/Pb^{207}$ ,  $Th^{232}/Pb^{208}$ ,  $Rb^{87}/Sr^{87}$ ,  $K^{40}/Ca^{40}$ , and  $K^{40}/Ar^{40}$ . An additional age can be found from the ratio  $Pb^{207}/Pb^{206}$ .

In order to measure the few millionths of a gram of an element present in the minerals, the method of isotope dilution has been used, the tracer isotopes having been made available by the Atomic Energy Commission. Ion-exchange resins are used for the separation and concentration of potassium, rubidium, and strontium. Solvent extraction, sometimes combined with special organic precipitants, is used for uranium, thorium, and lead. The ratio of the isotopes is determined with a mass spectrometer.

At the start of the year, the results of the co-operative program indicated that the ages calculated from the different decay

systems were of the same order of magnitude but did not always agree precisely when determined for a single mineral, for different minerals from the same rock, or from a suite of presumably geologically related minerals. In general, thorium-lead ages are the lowest, the ages from  $U^{238}/Pb^{206}$ ,  $U^{235}/Pb^{207}$ , and  $Pb^{207}/Pb^{206}$

essential agreement. It appears, however, that in order to make truly critical comparisons between the various schemes, extreme care must be taken in the selection of samples. The selection of rocks and minerals for analysis must be made under competent geologic guidance. It may well prove, as is now indicated, that certain

TABLE 5  
APPARENT AGES OF GRANITIC MINERALS

| GRANITE<br>LOCATION      | MINERAL   | CALCULATED AGE (MILLION YEARS) |                    |                     |              |
|--------------------------|-----------|--------------------------------|--------------------|---------------------|--------------|
|                          |           | $U^{238}/Pb^{206}$             | $U^{235}/Pb^{207}$ | $Pb^{207}/Pb^{206}$ | Rb/Sr        |
| Capetown, S. Africa..... | Zircon    | 330                            | 355                | $525 \pm 50$        | .....        |
|                          | Biotite   | ....                           | ....               | .....               | $820 \pm 40$ |
| Bagdad, Ariz. ....       | Zircon    | 630                            | 750                | $1190 \pm 70$       | .....        |
|                          | Muscovite | ....                           | ....               | .....               | 1600         |
| Uncompahgre, Colo. ....  | Apatite   | 1050                           | 1380               | $1810 \pm 160$      | .....        |
|                          | Biotite   | ....                           | ....               | 1650                | 1650         |
| Essonville, Ont. ....    | Zircon    | 1020                           | 1050               | 1060                | .....        |
| Ohio City, Colo.....     | Zircon    | 935                            | 1110               | $1480 \pm 110$      | .....        |

TABLE 6  
APPARENT AGES OF NORTH AMERICAN MICAS

| PEGMATITE<br>LOCATION                | MINERAL    | ANALYTICAL DATA        |                        |                                    | CALCULATED<br>AGE (MILLION<br>YEARS)<br>Rb/Sr |
|--------------------------------------|------------|------------------------|------------------------|------------------------------------|---|
|                                      |            | Rb <sup>87</sup> (ppm) | Sr <sup>87</sup> (ppm) | Sr <sup>87</sup> /Sr <sup>88</sup> |   |
| Bagdad, Ariz. ....                   | Lepidolite | $3280 \pm 60$          | $70 \pm 1$             | 18                                 | $1890 \pm 100$                                |
| Harding Mine, Dixon, N. M.....       | Lepidolite | $2180 \pm 50$          | $40.4 \pm 0.4$         | 30                                 | $1650 \pm 100$                                |
|                                      | Muscovite  | $1760 \pm 20$          | $31.8 \pm 0.6$         | 5                                  | $1600 \pm 100$                                |
| Brown Derby Mine, Ohio City, Colo... | Lepidolite | $6750 \pm 100$         | $137 \pm 2$            | > 100                              | $1780 \pm 100$                                |
| Bridger Mt., Bonneville, Wyo.....    | Lepidolite | $5150 \pm 120$         | $176 \pm 5$            | > 100                              | $3020 \pm 300$                                |

follow in order, and the rubidium-strontium ages are highest. The application of the potassium-calcium and potassium-argon decay schemes was still in the development stage.

During the past year, many of the earlier measurements have been repeated because of our increasing awareness of what constitutes an experimentally acceptable result. In some cases, the differences between the ages obtained from the several decay schemes have been lessened. Additional samples have, in some cases, given

minerals which appear to be unchanged since their formation may in fact have been selectively influenced in composition during some part of their existence. Only by a study of the associated minerals can we hope to evaluate this effect.

Table 5 presents a summary of the ages calculated for some minerals from five granites. In table 6 are shown the analytical data and ages calculated for micas from four pegmatites, two of which are believed to be related to two of the granites in table 5. The data in these tables are the



basis of the general conclusions stated here.

Last year we reported the results of some age measurements on South African lepidolites, two of which (Kinderzitt and Jakkalswater) were from Namaqualand, Cape Province. In table 7 these ages are compared with the results of measurements on two monazites from the same region. Uranium and lead were determined by isotope dilution, and the thorium was measured colorimetrically.

In addition to these efforts toward clarifying the age-determination picture, other work is in progress which relates to an-

face occurrences of dunite and from meteorites. We have examined several olivine bombs ejected from volcanoes, and measurements of the uranium and lead contents have been made, as well as of the isotopic ratios of the lead isotopes. Isotope-dilution measurements of the uranium content of the bombs gave values similar to earlier measurements made at the Laboratory on dunites and websterites, which are not of volcanic association. The uranium content is 0.007 part per million, which is as low as that of any measured terrestrial material.

TABLE 7  
APPARENT AGES OF NAMAQUALAND MINERALS

| LOCATION                       | MINERAL                        | CALCULATED AGE (MILLION YEARS)       |                                     |                                      |       |
|--------------------------------|--------------------------------|--------------------------------------|-------------------------------------|--------------------------------------|-------|
|                                |                                | Th <sup>232</sup> /Pb <sup>208</sup> | U <sup>238</sup> /Pb <sup>208</sup> | Pb <sup>207</sup> /Pb <sup>208</sup> | Rb/Sr |
| Steenkampskraal, Van Rhynsdorp | Monazite-spinel<br>intergrowth | 990                                  | 1090                                | ...                                  | ....  |
| Goodhouse area                 | Monazite                       | 900                                  | 930                                 | 880                                  | ....  |
| Kinderzitt                     | Lepidolite                     | ...                                  | ...                                 | ...                                  | 1210  |
| Jakkalswater                   | Lepidolite                     | ...                                  | ...                                 | ...                                  | 1100  |

other aspect of the earth's crust. The problem of the source of ultramafic rocks found at the surface of the earth has long been the subject of geological discussion. These rocks have been considered to be fragments of the material which underlies the crust of the earth and makes up the bulk of the earth's volume. The obvious difficulties of sampling have limited the direct analytical observation of the ultrabasic mantle to extrapolation from the few sur-

The bomb material contained 2 parts per million lead with an isotopic composition of  $206/204=19.5$ ,  $207/204=15.5$ , and  $208/204=39.7$ . These ratios indicate that the bomb material must have been derived from an environment containing more uranium than is present in the bombs today. The abnormally high value of  $206/204$  with respect to  $207/204$  appears to be real, was unexpected, and yields to no ready explanation.

## SYNTHETIC BASALT

*The system diopside—anorthite—water* (Yoder). Many of the important and unsolved problems in petrology are related to the crystallization of basalts. These problems include the origin of monomineralic rocks such as the pyroxenites and anorthosites, the formation of the layered laccoliths, and the kinds of primary basaltic magmas. Dry-melt studies have contributed to our understanding of the crystalli-

zation of basalts but have failed to provide solutions to these problems. New experiments, however, employing the principal components of basalts together with water, now promise to yield much more information.

A simple synthetic system that closely approaches basalt is the diopside—anorthite—forsterite system. The liquidus relations in this system were worked out in

the dry way earlier, and it was shown that the minimum temperature at which liquid occurs is  $1270^{\circ}\text{C}$ , well above the temperatures of most natural basaltic lavas. Although iron would be expected to lower the minimum melting temperature of this system appreciably, small amounts of water could also have a great effect. Basaltic magmas are believed to contain a small percentage of water. For this reason the system diopside—anorthite—forsterite

its dry position of 42 weight per cent anorthite to approximately 73 per cent. Accordingly, the eutectic temperature is lowered from  $1274^{\circ}\text{C}$  in the anhydrous system to approximately  $1095^{\circ}$  in the hydrous system. It is also of considerable interest that, at this water pressure and higher, anorthite, whose dry melting point is  $1550^{\circ}\text{C}$ , melts at a lower temperature in the presence of water vapor than does diopside, whose dry melting point is  $1391.5^{\circ}$ . Work is now under way with forsterite added, bringing us one step closer to a basaltic magma.

The completed portion of the study already has important bearing on several major problems. For example, the last liquid to crystallize is very rich in anorthite. This constitutes a strong argument that anorthosite, a highly refractory rock composed almost entirely of a basic plagioclase, can form from a liquid at temperatures believed to be readily attained in the earth's crust. The relations in the diopside—anorthite—water system also suggest a reasonable explanation for the rhythmically interlayered pyroxenites and anorthosites. Such rhythmically layered rocks are well known at Stillwater, Montana; in Aberdeenshire, Scotland; in the Bushveld region of South Africa, and in many other places. By varying the water vapor pressure over a critical range, it would be possible to shift the eutectic so that alternatively a pyroxene or a basic plagioclase could crystallize and settle out. Mechanisms for varying the water vapor pressure are readily available in a cooling magma; the simplest of these would involve fracture of the roof of the magma chamber with consequent release of dissolved water vapor, followed by further crystallization with a resultant increase in vapor pressure. Such a mechanism could well be cyclic.

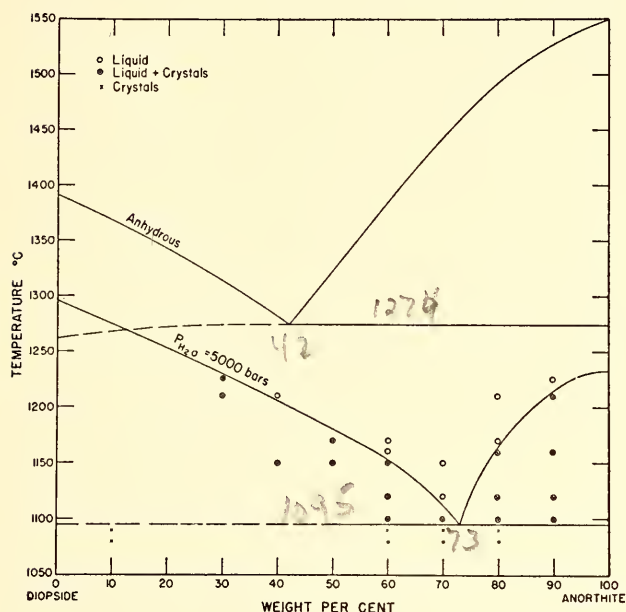


FIG. 4. The diopside—anorthite diagram (Osborn, Geophysical Lab. Paper No. 1069) and the preliminary diopside—anorthite—water diagram at 5000 bars water pressure.

—water is being investigated. The extreme conditions of temperature and pressure necessary to contain the water can now be obtained in a routine fashion with available apparatus.

The preliminary liquidus diagram for the diopside—anorthite—water system, a portion of the fundamental five-component system, is given in figure 4. At 5000 bars water vapor pressure the composition of the eutectic of the system is shifted from

## MAJOR MINERAL GROUPS

Study of major mineral groups has absorbed much of the investigative effort of the staff of the Geophysical Laboratory in

years past, and continues to do so today. There are two basic reasons for this continuity of interest and research.



One reason is the importance of precise knowledge of the physical conditions under which igneous and metamorphic rocks have formed. Only when the geologist has at hand a means of evaluating the temperature, rock pressure, and partial pressures of the several important volatiles under which rocks have crystallized can he hope to understand many of the major problems of geology. The only direct evidence we have of the conditions under which a given rock has crystallized lies in the nature and composition of its constituent minerals. For every equilibrium mineral assemblage there exists a stability field, defined primarily by the co-ordinates mentioned above. The experimental evaluation of the stability fields of phases in the major mineral groups gradually enlarges our quantitative knowledge of metamorphic and igneous activity, and gives a greater understanding of the physical conditions at depth in the earth's crust.

The second reason for the intensive study of major mineral groups is quite apart from a desire for knowledge of the physical conditions under which they have formed. Igneous activity and metamorphism are not static processes. Rather, they produce continuous changes in the bulk composition of the rocks they affect and in the chemistry and crystallography of the individual mineral phases comprising these rocks. The geologist cannot observe such processes in action, except in the case of volcanism. A knowledge of their nature and direction can come only through the examination of series of natural "quenched" products. Herein lies the basic reason for the detailed investigation of the compositional variation and crystallography of natural minerals.

A large body of information dealing with the chemistry and crystallography of silicates has been built up over the course of the past century. This knowledge is now being expanded more rapidly and effectively than at any time in the history of the science, largely owing to the development of new techniques in X-ray and

hydrothermal research. So great is the complexity of silicate systems that important and fundamental discoveries continue to be made.

The subjects under investigation at this Laboratory in the past year, and included here under the general heading "Major mineral groups," are diverse in character. They range from the single-crystal X-ray study of alkali feldspars to the synthesis of garnets under very high pressure. Although diverse in nature, these investigations share common aims and stem from the common interests outlined above.

The hydrothermal studies of the stability ranges of micas, amphiboles, and garnets all contribute to a better quantitative understanding of metamorphic processes. The investigations of the systems  $\text{NaAlSiO}_4$ — $\text{NaAlSi}_3\text{O}_8$ — $\text{H}_2\text{O}$  and  $\text{NaAlSiO}_4$ — $\text{KAlSiO}_4$  enlarge our knowledge of a group of minerals common in lavas and in certain plutonic rocks. New data have been obtained on perthitic alkali feldspars, and a new technique has been developed for their study. An important by-product of several of these investigations has been the construction of curves relating composition to X-ray spacings in certain solid solutions. These curves will be of great assistance in further studies of natural silicates.

#### AMPHIBOLES

Amphiboles are chemically a complex mineral group. The large number of different ion sites in the amphibole structure together with the manner in which the basic structural units, the  $\text{Si}_4\text{O}_{11}$  chains, are staggered permits extensive ionic substitution. In the calciferous amphiboles, which are the common amphiboles of igneous and metamorphic rocks, four substitutions are of particular interest from a stability viewpoint. Two of these are simple ion substitutions and two are more complex, coupled ion substitutions. The two simple substitutions are magnesium for ferrous iron and fluorine for the hydroxyl ion. The two coupled substitutions,

involving soda and alumina, are illustrated in figure 5, wherein they are shown as the ordinate and abscissa of a variation diagram. A selected group of analyses of calciferous amphiboles has been plotted in this diagram to show the range in composition encountered in natural amphiboles.

The field bordered by the joins tremolite—pargasite, pargasite—edenite, and

The principal objective of the investigation of stability relations in the calciferous amphibole group is the evaluation of the influence of these several substitutions on the stability of the amphibole structure. About 200 runs have been made to date, most of these as part of a reconnaissance through the system, designed to rough out stability regions and to find those starting materials which will yield the best reaction

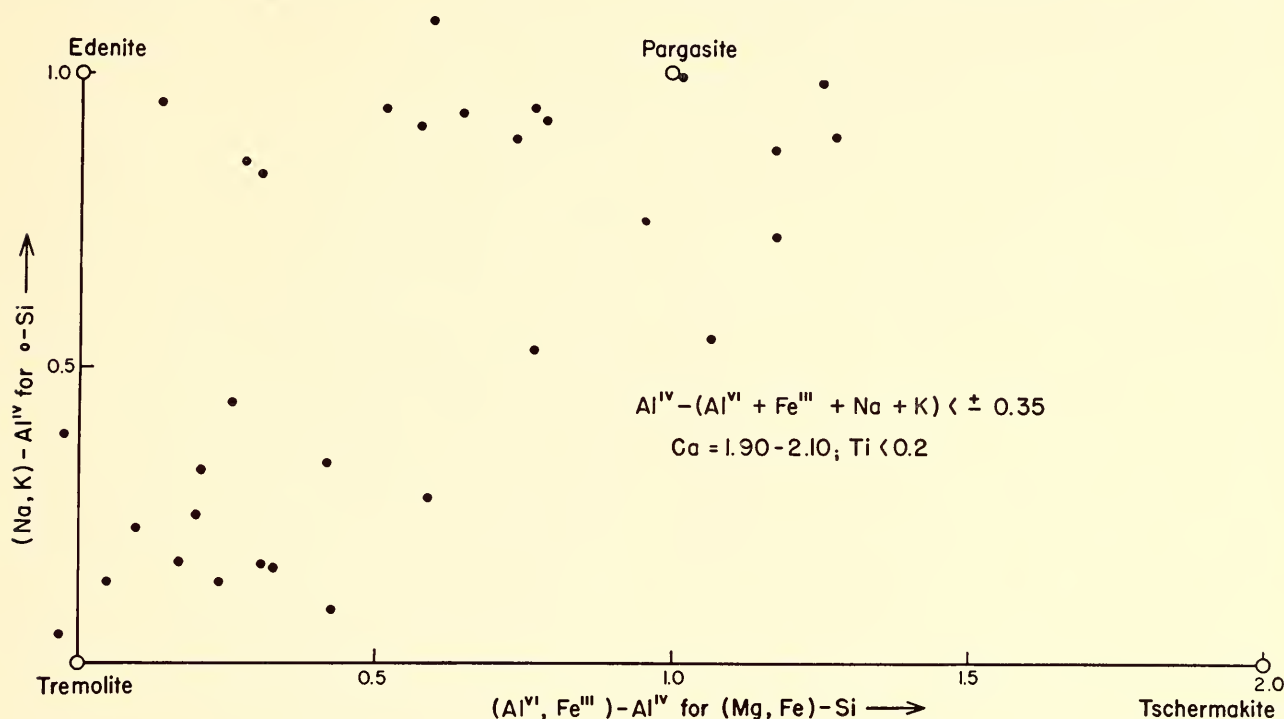


FIG. 5. Some analyses of calciferous amphiboles: tremolite,  $\text{oCa}_2(\text{Mg,Fe})_5\text{Si}_6\text{O}_{22}(\text{OH})_2$ ; edenite,  $\text{NaCa}_2(\text{Mg,Fe})_5\text{Al}^{\text{IV}}\text{Si}_7\text{O}_{22}(\text{OH})_2$ ; pargasite,  $\text{NaCa}_2(\text{Mg,Fe})_4\text{Al}^{\text{VI}}\text{Al}_2^{\text{IV}}\text{Si}_6\text{O}_{22}(\text{OH})_2$ ; tschermakite,  $\text{oCa}_2(\text{Mg,Fe})_3\text{Al}_2^{\text{VI}}\text{Al}_2^{\text{IV}}\text{Si}_6\text{O}_{22}(\text{OH})_2$ . The symbol o refers to a vacant lattice site. The Roman numeral superscripts for Al refer to co-ordination number, and those for Fe to the valence state.

edenite—tremolite is well populated. Tremolite is frequently found in nearly pure form in metamorphosed siliceous dolomites. Edenite can form in a similar environment if alkalis and alumina are present. The amphiboles of low-grade metavolcanics generally lie on the join tremolite—pargasite. The common amphibole in calcalkaline igneous rocks and high-grade metavolcanics approaches pargasite in composition.

There is a conspicuous absence of natural amphiboles with compositions near tschermakite. The reason for this absence may be found, in part, in considerations of stability under high pressure (see below).

rates. The time necessary to obtain an approach to equilibrium has been, on an average, about two weeks. Even on runs longer than a month, however, the reactions to form amphiboles almost never go to completion. The products obtained above about  $800^\circ\text{C}$  are, in all cases, mixtures of amphibole and high-temperature breakdown products. Below  $800^\circ$ , amphiboles can be grown, but they are always obtained in combination with other phases.

*Tremolite* (Boyd). A portion of the breakdown curve for tremolite is illustrated in figure 6. This curve has been drawn on the basis of the crystallization products of charges made up of glass or oxide mix-



tures. The breakdown temperature of natural tremolites agrees well with these data at a vapor pressure of 1000 bars. At pressures below 1000 bars, however, natural tremolites will not break down in any reasonable length of time, until the temperature is raised some 40° to 50° C above this curve.

Both tremolite and its breakdown products—enstatite, diopside, and quartz—crystallize well in the vicinity of the curve at water pressures of 500 to 1000 bars. Below 500 bars, metastable assemblages, such as quartz and cristobalite together, and a single pyroxene solid solution make their

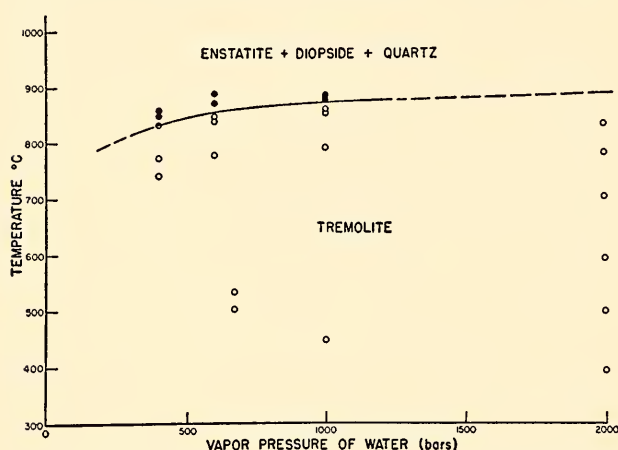


FIG. 6. The univariant equilibrium curve for the reaction tremolite  $\rightleftharpoons$  3 enstatite + 2 diopside + quartz + vapor.

appearance. No tremolite has been grown below 400 bars, even in runs of more than a month.

*Pargasite and edenite* (Boyd). Pargasite and edenite have been synthesized hydrothermally in the range 800° to 900° C and 400 to 1000 bars. Their optical properties are in accord with those of the corresponding natural amphiboles. Reconnaissance data indicate that the edenite stability field will extend to about 25° C above the tremolite curve.

*Tschermakite* (Boyd). Tschermakite has not been obtained in any hydrothermal runs at pressures of 2000 bars or less. The aluminous phase obtained by crystallizing a glass of tschermakite composition hydrothermally above about 500° is anorthite.

The co-ordination of aluminum in anorthite is fourfold; in tschermakite the aluminum would be half in fourfold co-ordination, half in sixfold. Theoretically, the higher co-ordination of aluminum is favored by high pressure. Accordingly, the investigation of tschermakite was extended to extreme pressures. With the co-operation of Yoder, a glass of tschermakite composition and a glass midway on the join tremolite—tschermakite were run at 800° and 10,000 bars. The glass midway on the tschermakite—tremolite join crystallized almost completely to amphibole; the glass of tschermakite composition crystallized partly to amphibole. It is apparent that, under very high pressures, amphiboles can be made whose composition extends at least halfway between tremolite and tschermakite, and possibly farther. These aluminous amphiboles do not appear to have a stability field at low pressures. The failure to obtain them at low pressures cannot, however, be taken as a certain indication that they have no stability field in this region.

*Fluoramphiboles* (Boyd). An investigation of the fluoramphiboles has been carried out along with the hydrothermal work. Fluoramphiboles can be readily crystallized by heating charges in sealed platinum tubes at temperatures a little over 1000° C. Tremolite, edenite, pargasite, and tschermakite have all been made with fluorine in this manner. As was found in the hydrothermal work, the amphiboles obtained are always mixed with other phases. More complete reaction can, however, be obtained with fluorine than with water. The effect of the substitution of fluorine for the hydroxyl ion is a very marked increase in the stability range of an amphibole. From a stability viewpoint, this substitution is by far the most important yet examined.

It is apparent from the data on hand that tremolite will be stable to high metamorphic grades, even under very low partial pressures of water, in assemblages of minerals with which it will not react. Dis-

appearance of tremolite at lower grades during progressive metamorphism is due to its reaction with other phases, usually carbonates. The temperature at which such reactions take place will be a complex function of the  $\text{CO}_2$  pressure, as well as of the  $\text{H}_2\text{O}$  pressure. The Mg-pargasite, which is an approximation to the average igneous or metamorphic hornblende, can be stable well into the magmatic range.

### MICAS

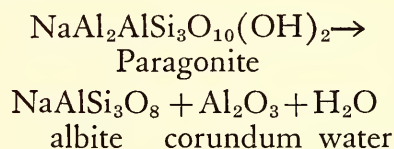
Micas are among the most common rock-forming minerals. They show great compositional variation and can form under a wide range of physical and chemical conditions. They are found in natural environments ranging from sediments to lavas. In an effort to rationalize quantitatively the mass of field evidence accumulated over many years, the Laboratory began hydrothermal and X-ray work on the micas two years ago. We have been able to illuminate such questions as differences of occurrence and behavior of dioctahedral (muscovite) and trioctahedral (phlogopite) micas, and have gained understanding of the relation of the polymorphs. In the past our efforts have been concentrated on members of the mica group which contain potassium in the alkali position. These potassium-bearing micas are the most abundant and also the best-known types of mica. The sum of our present knowledge of them, based on optical properties, crystal structure, field relations, and the new hydrothermal studies, enables us to understand and explain many rock-forming processes involving these micas.

It has been known that other ions of comparable size can proxy for potassium in silicate lattices—of the major constituents, notably sodium and calcium. These three ions have diverging ionic properties—in the case of calcium, even different charge—and it is to be expected that substitution will be possible only under limited circumstances. In the case of feldspars much had been learned about this problem

with the aid of hydrothermal studies. We therefore extended our work on the potassium-bearing micas to include the sodium mica paragonite and the calcium mica margarite.

*Paragonite* (Eugster, Yoder). Investigation of the sodium mica proved to be exceedingly important in several respects. Paragonite as a rock-forming mineral occurs always in very small flakes. It is not possible optically to distinguish these flakes from fine-grained muscovite (“sericite”), and they are therefore included in the group name “white micas.” All metamorphic reactions leading to white micas must involve other minerals with sodium and potassium as main constituents. We can deduce the nature of these reactions only if we know the fate of the alkali metals. Chemical analysis on these fine-grained materials can give us no more than the bulk composition. It became apparent during our work on paragonite that a combination of hydrothermal experiments and detailed X-ray studies on natural material is very fruitful. We now have a good picture of the mineral assemblages to be expected during the recrystallization of the fine-grained mica schists.

Paragonite was synthesized from a variety of mixtures of the proper composition. The reaction of the breakdown of paragonite is as follows:



The position of the univariant curve representing this reaction is given by the following points:

| Pressure<br>(atm) | Temperature<br>(°C) |
|-------------------|---------------------|
| (1)               | (395)               |
| (68)              | (519)               |
| 340               | 580                 |
| 680               | 610                 |
| 1020              | 625                 |
| 2040              | 660                 |



The two points enclosed in parentheses were calculated using the Nernst approximation. Figure 7 summarizes the results of the experiments. For purposes of comparison, the curve for the breakdown of muscovite, which lies about  $40^\circ$  higher, is also given.

Polymorphism plays an important role in all the micas. Single crystals of suitable

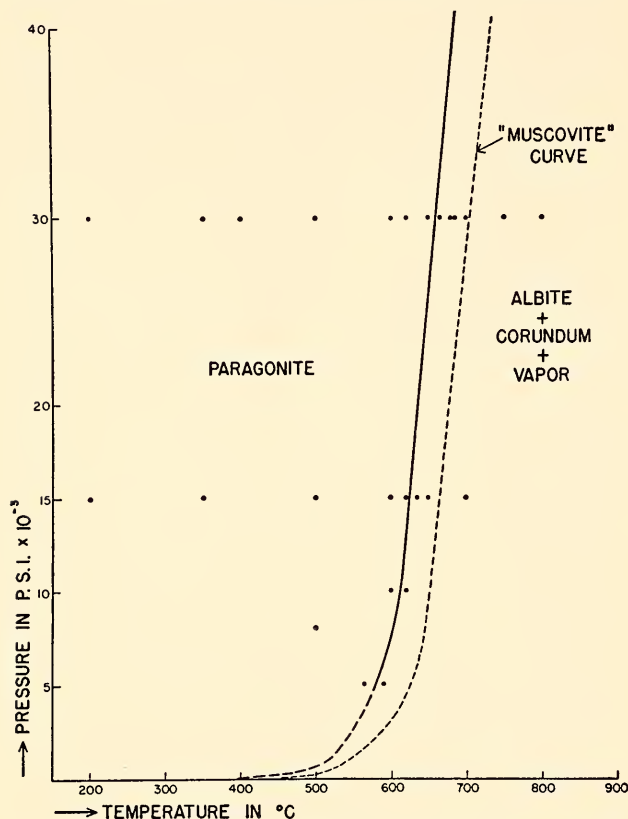


FIG. 7. Pressure-temperature curve for the reaction  $\text{paragonite} \rightarrow \text{albite} + \text{corundum} + \text{vapor}$ . Each dot represents the pressure and temperature conditions of one or more runs. The univariant curve for the reaction  $\text{muscovite} \rightarrow \text{sanidine} + \text{corundum} + \text{vapor}$  is given for comparison.

size for investigation of this problem were found from two localities: Chester, Pennsylvania, and Postmasburg District, South Africa. Weissenberg photographs showed the paragonite ("ephesite") from South Africa to be a two-layer monoclinic (2M) modification.

In the course of the hydrothermal experiments on synthetic mixtures, two different X-ray patterns were obtained, which could be assigned to two different modi-

fications of paragonite, namely a one-layer monoclinic (1M) and a two-layer monoclinic (2M) modification. From the experimental work the following conclusions can be drawn as to the relative stability of these two polymorphs.

One-layer monoclinic paragonite grows in runs of short duration at all temperatures and pressures within the stability field. At temperatures between  $400^\circ$  and the upper stability limit this modification is slowly converted into the two-layer monoclinic modification. From these results it can be deduced that in the region between  $400^\circ$  and the upper stability limit the two-layer monoclinic form represents the more stable modification. This view is supported by the fact that the X-ray patterns of all natural paragonites known today are identical with those of the two-layer monoclinic modification.

Natural paragonite has been reported from only a few localities. Identification was based solely on chemical analysis, and, even in the purest of these paragonites, the sodium content was found to be 1 to 2.5 per cent lower than the theoretical value. The low sodium content has usually been attributed to solid solution with muscovite.

From the experimental work it could be deduced that paragonite must be a much more common mineral than appears from the reported occurrences, since its stability field is almost as large as that of muscovite; and investigation of dozens of fine-grained mica schists by X-ray methods has borne out this suspicion. In many specimens it was observed that muscovite and paragonite occurred side by side, yielding the pattern of a mechanical mixture. This was immediate proof for the absence of a continuous series of solid solution for a wide range of conditions. The solid solution is very limited, and its exact extent is now being determined with a large suite of samples.

Most published chemical analyses have been performed on mixtures of muscovite and paragonite and are not helpful, there-

fore, in evaluating the limits of solid solution. Today only five occurrences of paragonite free of muscovite are known by X-ray investigation. They are: Alpe Sponda, Switzerland; Pizzo Forno, Switzerland; Fenestrella, Italy; Chester, Pennsylvania; and Postmasburg, South Africa. Most other specimens labeled paragonite are either mixtures of muscovite and paragonite or pure muscovite. Table 8 gives a summary of the results of X-ray investigation of some specimens obtained from the U. S. National Museum.

onite formed an exceedingly fine, flaky mass.

The most important result of these studies is the discovery that paragonite is an important rock-forming mineral with a stability field close to that of muscovite, and that it is found in many metamorphic rocks containing fine-grained micas.

A summary of the relations of the mineral assemblages in metamorphic rocks containing dioctahedral micas and feldspars is given in figure 8.  $\text{Al}_2\text{O}_3$  lies in the same plane with albite, paragonite,

TABLE 8  
X-RAY INVESTIGATION OF SO-CALLED PARAGONITE SPECIMENS

| U. S. Nat.<br>Mus. no. | Locality                    | Name        | Mineralogy             |
|------------------------|-----------------------------|-------------|------------------------|
| R 4414.....            | Mte Campione, Switzerland   | Paragonite  | Paragonite + muscovite |
| R 4415.....            | Pregratten, Tirol           | Pregrattite | Muscovite + paragonite |
| R 4416.....            | Fenestrella, Italy          | Cossaite    | 2M paragonite          |
| R 4417.....            | Borgofranco, Italy          | Cossaite    | 2M muscovite           |
| C 3033.....            | Mte Campione, Switzerland   | Paragonite  | Paragonite + muscovite |
| C 3661.....            | Miask, Russia               | Paragonite  | Muscovite + paragonite |
| C 3662.....            | Unionville, Chester, Penna. | Euphyllite  | 2M muscovite           |
| 12423.....             | Unionville, Chester, Penna. | Euphyllite  | Muscovite + paragonite |
| 93560.....             | Ivigut, Greenland           | Ivigite     | 1M + 2M muscovite      |

In order to obtain a real picture of the frequency of occurrence of paragonite, some 100 low-grade metamorphic schists containing white micas as a major constituent were investigated. The samples, collected by Arden Albee, represent a good coverage of the Lincoln Mountain and Hyde Park quadrangles in Vermont. Thirty-seven per cent of the specimens showed paragonite, from trace to appreciable amounts. Never more than 60 per cent of the white mica consisted of paragonite, the rest being muscovite. Some specimens carried pyrophyllite as well as muscovite and paragonite. Paragonite is often found in assemblages with kyanite or chloritoid.

Staining of some of the sections for potassium made it possible to differentiate between the two micas in thin sections. In the examples studied, a definite hiatus in the grain size was observed, muscovite being fairly coarse-grained, whereas parag-

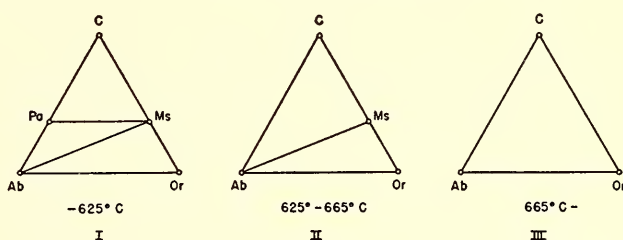


FIG. 8. Sequence of the mineral assemblages in the triangle  $\text{Al}_2\text{O}_3\text{—K}_2\text{O}\cdot\text{Al}_2\text{O}_3\cdot 6\text{SiO}_2\text{—Na}_2\text{O}\cdot\text{Al}_2\text{O}_3\cdot 6\text{SiO}_2$  of the system  $\text{Na}_2\text{O—K}_2\text{O—Al}_2\text{O}_3\text{—SiO}_2$  at 1000 bars water pressure. The relative positions of muscovite and paragonite are displaced to clarify the picture. C, corundum ( $\text{Al}_2\text{O}_3$ ); Ab, albite ( $\text{Na}_2\text{O}\cdot\text{Al}_2\text{O}_3\cdot 6\text{SiO}_2$ ); Or, orthoclase ( $\text{K}_2\text{O}\cdot\text{Al}_2\text{O}_3\cdot 6\text{SiO}_2$ ); Ms, muscovite ( $\text{K}_2\text{O}\cdot 3\text{Al}_2\text{O}_3\cdot 6\text{SiO}_2\cdot 2\text{H}_2\text{O}$ ); Pa, paragonite ( $\text{Na}_2\text{O}\cdot 3\text{Al}_2\text{O}_3\cdot 6\text{SiO}_2\cdot 2\text{H}_2\text{O}$ ).

muscovite, and orthoclase. In nature there is usually enough  $\text{SiO}_2$  available to form kyanite, andalusite, sillimanite, or pyrophyllite. For case I (fig. 8) the most common assemblages are paragonite + muscovite + pyrophyllite or kyanite, and musco-



vite + albite + orthoclase. Muscovite + paragonite + albite has been found in several specimens, but appears to be rarer. The assemblages paragonite + albite + orthoclase and paragonite + muscovite + orthoclase are theoretically possible, but the tie line in case I has been drawn from albite to muscovite because these assemblages have never been found. Their occurrence would mean that the reaction paragonite + orthoclase  $\rightleftharpoons$  albite + muscovite takes place at some temperature lower than 625°.

*Margarite* (Eugster, Yoder). In addition to paragonite, the calcium mica margarite has also been studied. The growth problems proved to be even greater than those for paragonite, and the time required for reaching equilibrium was found to be too long to make the determination of the P-T curve for the reaction margarite  $\rightarrow$  anorthite + corundum + vapor practicable. At 30,000 pounds per square inch, margarite was found to grow at 500° C and below, but did not appear at 600° C and higher. Work is continuing on the join margarite—paragonite.

#### FELDSPARS

The importance of the feldspars in geologic and petrologic theory cannot be overemphasized. They are ubiquitous constituents of igneous and metamorphic rocks, and mirror, in their complex crystallographic and optical variation, changes in their physical environment to a degree probably not equaled by any other group of minerals. Consequently, the feldspars have been the object of many investigations in the past. The development of new, rapid X-ray methods has now led to further understanding of the complex relationships existing among these minerals.

Alkali feldspars produced by laboratory syntheses have been found to have properties similar to those of natural feldspars derived from high-temperature environments. Thus far it has proved impossible to synthesize feldspars with properties like

those of natural varieties from low-temperature environments. The differences between high- and low-temperature forms are believed to be due to the different distribution of tetrahedrally co-ordinated silicon and aluminum in their respective structures. The highly ordered arrangement of silicon and aluminum in the low-temperature forms apparently cannot be developed in the length of time available to a laboratory investigator. Hence, it is necessary to undertake a detailed study of natural feldspars in order to understand the subsolidus relations in this group.

The alkali feldspars can be divided into four series: high sanidine—high albite, sanidine—anorthoclase—high albite, orthoclase—low albite, and microcline—low albite. A systematic single-crystal X-ray and optical study of analyzed mineral specimens falling in the last three series has been made. The specimens were obtained from many parts of the world, and it was not possible to correlate the structures of the perthitic intergrowths with their petrological history. We have, however, obtained a good idea of the relations both in and between these three series, and on this basis have started a systematic study of feldspars from the same petrographic province. This work was made possible by the development of a simple method, both rapid and accurate, for determining the nature of the unmixed phases in the cryptoperthites.

*A single-crystal X-ray technique for the examination of alkali feldspars* (Smith, MacKenzie). Single-crystal techniques can reveal structural features not accessible by the powder X-ray methods. The single-crystal techniques used so far, however, have been too complicated and time-consuming to be used on large numbers of crystals. A simple method therefore was developed, which uses only the simplest single-crystal camera, the oscillation camera, and this method is hardly more difficult than operating a universal-stage microscope. The total time required for the study of each crystal is about a day. The

photographs are easy to interpret, and measurements of the lattice angles  $\alpha^*$  and  $\gamma^*$  of the twinned phases can be made in half an hour with high accuracy.

The nature of the unmixed phases can be accurately and definitely obtained from these two angles. In addition, a simple X-ray method for determining the composition of homogeneous single crystals is being developed and will soon be brought into routine operation. Before the single crystal is mounted in the X-ray camera, its  $2V$  is measured on a universal-stage microscope. The four measurements of  $2V$ , composition,  $\alpha^*$ , and  $\gamma^*$  provide a powerful tool for the interpretation of the structures of the feldspars, as is shown in the next section.

The details of the X-ray method are as follows. The crystal is mounted in the oscillation camera with its  $b$ -axis parallel to the oscillation axis. The crystal is oscillated through  $15^\circ$  with the (001) face parallel to the X-ray beam in the center of the oscillation. The soda and potash phases in the feldspar give two sets of reflections, which may be easily recognized, for the potash reflections are closer to the center of the photograph. The symmetry of the phases may be readily determined by visual comparison of the intensities. The twinning of the triclinic phases results in pairs of reflections which are parallel to the layer lines for pericline twinning and parallel to the row lines for albite twinning. In many cases complex superstructures are formed which produce arrays of reflections on the photographs. Examination of the diffuseness of the reflections and of the streaks which often connect the reflections provides information on the nature of the lamellae and on the development of the unmixed phases. The lattice angles  $\alpha^*$  and  $\gamma^*$  are obtained by measuring the displacement of the paired reflections arising from the twinning, and converting these measurements by the aid of charts.

*Orthoclase and sanidine perthites* (MacKenzie, Smith). From single-crystal X-ray

oscillation photographs of a number of analyzed alkali feldspars from both high- and low-temperature geologic environments, a systematic study of the lamellar structure of these feldspars has been made. The perthites generally consist of two phases, one soda-rich and one potash-rich. Specimens have been found, however, consisting of one potash phase and two soda phases or two potash phases and one soda phase. Measurements of the reciprocal lattice angles  $\alpha^*$  and  $\gamma^*$  of the soda phases permit a distinction to be made between high- and low-temperature forms, and it is believed that the composition of the soda phase may be determined approximately from these angles.

By plotting  $\alpha^*$  against  $\gamma^*$  for both high- and low-temperature forms of soda-rich feldspars, diagrams have been constructed to compare the reciprocal lattice angles of the soda phases of perthites with those of individually crystallized soda feldspars. In figure 9 the values of the reciprocal lattice angles of the soda phase of eight orthoclase-micropertites are plotted, and, with one exception, these fall near low-temperature albite. The departure of these points from that representing low-temperature albite is accounted for by the solid solution of potash feldspar or lime feldspar in the soda phase. Feldspars with a high anorthite content in their bulk composition have a soda phase whose reciprocal lattice angle  $\gamma^*$  is less than that of low-temperature albite, almost certainly owing to lime feldspar in solid solution in the soda phase. The effect of solid solution of potash feldspar in the soda phase is to increase the value of  $\alpha^*$ , but only a very limited amount of potash feldspar can be retained in low-temperature albite.

The specimen whose soda phase is represented by the point *E* (fig. 9) is a potash-rich feldspar of undoubtedly low-temperature origin, yet the reciprocal lattice angles of the soda phase are close to those of a high-temperature feldspar of composition near  $\text{Or}_{25}\text{Ab}_{75}$ . It is probable that the soda phase is in the high-temperature form be-



cause it contains more potash in solid solution than can be accommodated in the low-temperature form.

Figure 10 shows the plot of the reciprocal lattice angles of the soda phase of three high-temperature feldspars belonging to the sanidine—cryptoperthite series. These points (5, 10, and 12) fall near the expected values for high-temperature feldspars with about 15 to 25 per cent potash feldspar in solid solution. In the same diagram the

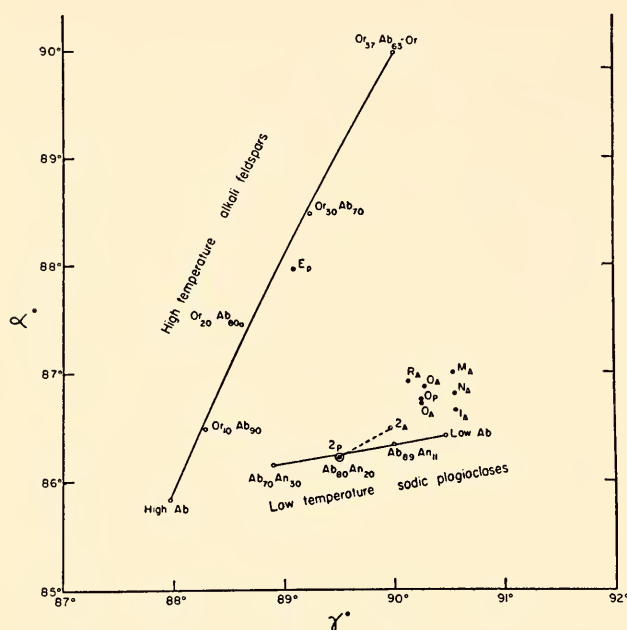


FIG. 9. Reciprocal lattice parameters  $\alpha^*$  and  $\gamma^*$  of homogeneous soda-rich alkali feldspars, and the soda phase of eight low-temperature perthites. The suffixes *A* and *P* indicate the type of twinning (albite or pericline) shown by the soda phases.

reciprocal lattice angles of the soda phases of two feldspar crystals from the Beinn an Dubhaich granite, in the island of Skye, are plotted, and these points are denoted by the letter *S*. The alkali feldspars from this granite have been found to vary in the value of  $2V$  in different crystals from the same specimen. This variation has been attributed to partial inversion from sanidine-perthite to orthoclase-perthite. Single-crystal X-ray studies of two of these crystals with  $2V=45^\circ$  and  $54^\circ$  show that there are two soda phases in each crystal, one near low-temperature albite and the other comparable with a high-temperature soda-

rich feldspar. The variation in optic axial angle is due to the relative proportions of the high- and low-temperature soda phases; thus the crystal with  $2V=45^\circ$  has a greater proportion of high-temperature soda feldspar.

A much more detailed study of the feldspars in this granite has now been started, and it is proposed to extend this study to

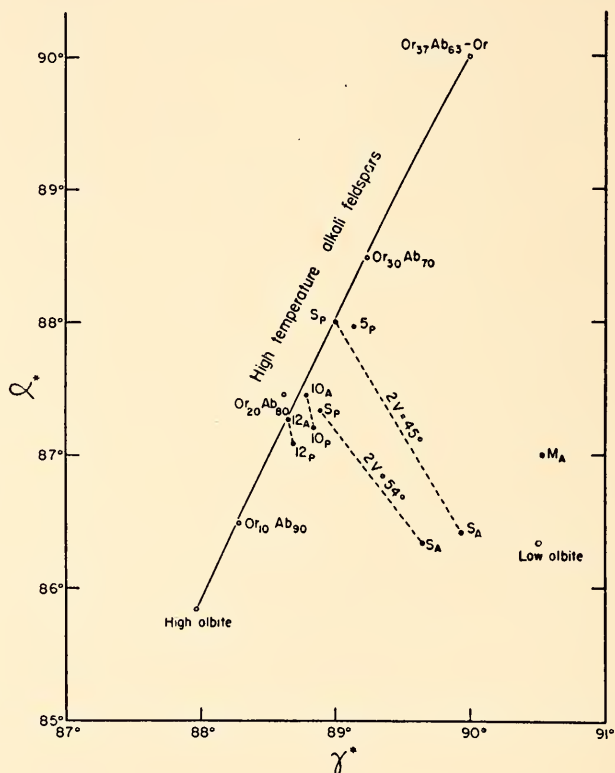


FIG. 10. Reciprocal lattice parameters of the soda phase of three high-temperature perthites. The suffixes *A* and *P* denote the type of twinning (albite or pericline) shown by the soda phases. *S*, plots of the reciprocal lattice angles of the soda phase of two feldspar crystals from the Beinn an Dubhaich granite, island of Skye. (See text for further explanation.)

other granites from the British Tertiary province and to older granites.

**Microclines** (MacKenzie). Triclinic potash feldspar can readily be distinguished from the monoclinic form by the use of X-ray powder diffraction patterns. In addition, microclines differ in their obliquity or degree of departure from monoclinic symmetry. From single-crystal and powder X-ray studies of potash-rich alkali feldspars it has been found that there are two differ-

ent ways in which monoclinic and triclinic feldspars coexist: (a) the monoclinic material is associated with triclinic material having a fixed and fairly large departure from monoclinic symmetry, or (b) the monoclinic material is associated with triclinic material which shows all gradations in its deviation from monoclinic symmetry.

In three new chemical analyses of maximum microclines (maximum microcline is the term proposed to denote a microcline having the maximum obliquity so far found, i.e.,  $\alpha=90^\circ 41'$ ,  $\gamma=87^\circ 40'$ ) from which most of the perthitic albite had been separated by repeated centrifuging in heavy liquids, the amount of soda feldspar in solid solution was less than 10 per cent. No maximum microclines have been found to have more than 10 per cent soda feldspar in solid solution, although microclines with less than the maximum obliquity may have as much as 15 to 20 per cent. Unfortunately, it is not possible from the obliquity of a microcline to determine the composition of the potash phase.

It is believed that the existence of the microcline lattice, and to some extent also the obliquity of the lattice, is controlled by the chemical composition of the potash phase. The composition of the potash phase is, in most cases, dependent on the extent to which exsolution of soda feldspar has proceeded.

*The crystal structure of the feldspars* (Smith). It is believed that the high-temperature forms of the feldspars contain disordered silicon and aluminum atoms, whereas the low-temperature forms contain highly ordered arrangements of these atoms. In order to investigate this hypothesis, a program of work is in progress to determine accurately the crystal structure of the end members. As is shown in a later section ("The Si-O and Al-O distances"), measurement of the size of the oxygen tetrahedra permits the determination of the contents of the tetrahedra and hence the determination of the ordering.

High-temperature sanidine has been shown by others to contain disordered alu-

minum and silicon atoms. The structure determination of orthoclase is being undertaken. A complete set of intensities for the three principal zones has been collected, and these will be transformed into electron density maps. The positions of the aluminum and silicon atoms can be deduced from the positions of the atoms on the maps, thus providing a measure of the ordering.

*Lattice parameters of the plagioclases* (Smith). Measurements of lattice parameters of the sodic plagioclases have been made. These measurements have been extended to a composition of An<sub>60</sub>. The remaining compositional region from An<sub>60</sub> to An<sub>100</sub> is now under study.

*The Si-O and Al-O distances* (Smith). The positions of the silicon and aluminum atoms in a crystal structure can at present be determined only by measuring the sizes of the oxygen tetrahedra which contain the silicon and aluminum atoms. Measurements of (Si, Al-O) for paracelsian and high-carnegieite, reported earlier, suggested that the previously accepted values for Si-O and Al-O were incorrect. In view of the large amount of work being expended on the determination of the positions of silicon and aluminum atoms in the feldspars, it was thought desirable to review all the measured Si-O and Al-O distances in order to obtain the best values.

Accordingly, a critical review of the measurements of Si-O and Al-O recorded in the literature was made, resulting in the values Si-O =  $1.60 \pm 0.01$  Å and Al-O =  $1.78 \pm 0.02$  Å. These values are in good agreement with those given in Year Book No. 52, and they differ considerably from the values 1.62 and 1.72 used by previous workers. The new values show that the problem of distinguishing aluminum from silicon atoms is not so difficult as was at first thought. Using the latest methods, it should be possible to determine the relative distribution of aluminum and silicon in the structure to an accuracy of about 5 per cent.



## FELDSPATHOIDS

The feldspathoids are a group of minerals chemically allied to the alkali feldspars but having a higher ratio of alkalis to silica. The most important rock-forming feldspathoids are nepheline and leucite, and the rocks in which they occur are known as alkaline rocks. The interest in these rocks shown by petrologists and mineralogists alike is out of all proportion to their frequency of occurrence, most of the alkaline rock masses being limited to relatively small areas. Their relative enrichment in rarer elements and their extreme mineralogical variability, however, give them considerable importance, and the problem of the origin of such rocks is one on which petrologic opinion is divided. During the past year several experimental investigations have been directed to the problems presented by this group of minerals and the rocks in which they occur.

The system  $\text{NaAlSiO}_4\text{—KAlSiO}_4\text{—SiO}_2$  is of fundamental importance in the interpretation of the genesis of acid-alkaline and subalkaline rocks. The silica-poor portion of this system can be called the nepheline syenite system, inasmuch as nepheline syenites are composed chiefly of nepheline, potash feldspar, and albite. The studies of the equilibrium relations in the systems  $\text{KAlSiO}_4\text{—NaAlSiO}_4$  and  $\text{NaAlSiO}_4\text{—NaAlSi}_3\text{O}_8\text{—H}_2\text{O}$  (reported below) are preliminary to the investigation of the nepheline syenite system in the presence of water vapor.

*The system  $\text{KAlSiO}_4\text{—NaAlSiO}_4$*  (Smith). During the course of the past year, work on this system has continued and a new phase has been encountered. This phase has the composition  $\text{KAlSiO}_4$  and was produced "dry" at  $1075^\circ\text{C}$  in a flux of KF by Bowen. The X-ray pattern shows that it is hexagonal, with  $a=5.180 \pm 0.002$  and  $c=8.559 \pm 0.004$  Å. The lattice parameters are close to those of kalsilite, but the intensities of equivalent reflection are quite different. There is, however, a striking resemblance between the powder

patterns of the new phase and those of natural kaliophilite. The kaliophilite pattern, which is based on the large 27 Å hexagonal cell, contains many more lines than the pattern of the synthetic phase, but includes lines which have the same position and intensity as the lines of the synthetic phase. These lines are those which fit a subcell obtained by reducing the  $a$ -axis of natural kaliophilite by  $3\sqrt{3}$ . The correspondence between the intensities of the reflections strongly suggests that the structure of the new phase is very similar to the average structure of kaliophilite. It is well known that disordered structures tend to form first. When these structures are held for a long time at a lower temperature, they then tend to order to a more stable configuration. Accordingly, it is suggested that kaliophilite first grows with the simple structure of the synthetic material, which in the course of time transforms into the structure of natural kaliophilite. The new synthetic phase has been termed synthetic kaliophilite. All attempts to make crystals identical with natural kaliophilite have failed.

The lattice parameters of the  $O_2$  (high-orthorhombic) phase of  $\text{KAlSiO}_4$  were obtained by indexing its powder pattern. They are  $a=8.892$ ,  $b=10.468$ , and  $c=8.547$  Å. Unmixing of nepheline-kalsilite solid solutions occurs very rapidly, and difficulty was experienced in determining the top of the miscibility gap because it was difficult to obtain a good quench. This problem was solved by X-raying the crystals at high temperatures and determining the temperature at which the nepheline and kalsilite mixture transformed into a single phase. By this means it was found that the top of the solvus was at  $1075^\circ \pm 25^\circ\text{C}$ . This result agreed with those of runs in which crystals were grown near the top of the solvus in the dry way, quenched in mercury, and then identified by X-ray methods at room temperature.

Above the unmixing solvus, kalsilite with a 5 Å  $a$ -axis is stable at the potassic

end, and nepheline with a 10 Å *a*-axis is stable at the sodic end. Powder records were taken at temperatures just above the solvus in order to determine how the 5 Å structure changed into the 10 Å structure. It was found that the 5 Å structure is stable from Ne<sub>0</sub> to about Ne<sub>20</sub>, whereas the 10 Å structure is stable from Ne<sub>30</sub> to Ne<sub>100</sub>. As the composition changed it was found that the 10 Å structure gave X-ray patterns more and more similar to those of the 5 Å structure as the composition Ne<sub>20</sub> was approached. It was not possible to determine whether the 10 Å structure changes continuously into the 5 Å structure or whether there is a discontinuity.

Attempts have been made to determine the phase relations at high temperatures for the potassic end of the system. This region was found to be very complex, and complete success was not attained. We have found that the orthorhombic KAlSiO<sub>4</sub> phase is stable from 840° C up to the liquidus; we have not, however, been able to define the stability fields of the *H*<sub>4</sub> and *O*<sub>2</sub> phases. It seems likely that growth occurs on quenching, giving confusing results. Attempts to X-ray the crystals at very high temperature (1300° to 1500° C) failed because large amounts of leucite were produced. This showed that alkali atoms had been lost from the charges, and, in view of this change in composition, reliable results could not be obtained.

After our demonstration of the unmixing solvus between nepheline and kalsilite, confirming evidence was given by Dr. Th. G. Sahama, who described perthitic intergrowths of nepheline and kalsilite in a specimen obtained from a lava flow in East Africa. Comparison of the composition of the nepheline from the specimen with the data from the synthetic crystals suggests a temperature of at least 700° C for the lava flow.

Work on this system has been carried out in collaboration with Dr. O. F. Tuttle of Pennsylvania State University. Dr. Th. G. Sahama of the University of Helsinki has also collaborated in an X-ray

study of the composition of natural nephelines.

*The system NaAlSiO<sub>4</sub>—NaAlSi<sub>3</sub>O<sub>8</sub>—H<sub>2</sub>O* (MacKenzie). An investigation of the equilibrium relations along the join NaAlSiO<sub>4</sub>—NaAlSi<sub>3</sub>O<sub>8</sub> under a water vapor pressure of 1000 bars has been made. These relations have proved to be similar to those found in the dry-melt work in this system. The minimum melting composition and temperature under a water vapor pressure of 1000 bars has been found to be Ab<sub>72</sub>Ne<sub>28</sub> at 870° ± 5° C, compared with Ab<sub>76</sub>Ne<sub>24</sub> at 1068° ± 5° C in the "dry" system.

The limit of nepheline solid solutions richer in silica than NaAlSiO<sub>4</sub> was found to be about Ne<sub>75</sub>Ab<sub>25</sub> at 750° C. The refractive indices of nephelines decrease appreciably with increase in their silica content, but only very slight intensity differences were detected in the X-ray powder diffraction patterns. Pure nepheline has refractive indices very close to those of albite, so in a fine-grained preparation it would be extremely difficult to detect small amounts of one phase admixed in the other. Because of the lowering of the refractive indices of nepheline with increase in its SiO<sub>2</sub> content, however, it is possible to detect under the petrographic microscope small hexagonal and rectangular crystals of nepheline in a preparation of composition Ab<sub>95</sub>Ne<sub>5</sub> crystallized at 700° C. At this temperature, then, the amount of solid solution of nepheline in the albite phase is extremely small.

Analcite (NaAlSi<sub>2</sub>O<sub>6</sub>·H<sub>2</sub>O) and jadeite (NaAlSi<sub>2</sub>O<sub>6</sub>) are possible phases in this system. Analcite has not been encountered in this work, since investigations were not carried out below 600° C and analcite is known to be stable only below 560° C at a water vapor pressure of 1000 bars.

#### OLIVINES

*The forsterite-fayalite series* (Yoder). The olivines are constituents of many volcanic and plutonic rocks. The petrologist



concerned with the origin of such rocks has need of a convenient and accurate method of estimating the chemical composition of members of the olivine group. A new X-ray method of determining their composition has now been developed to a practical stage. The composition of an olivine can be estimated by this method to within  $\pm 2$  mole per cent in less than 20 minutes per specimen.

The determinative curve in figure 11 is based on accurate measurement of the 130 spacing of the crystals, using an internal

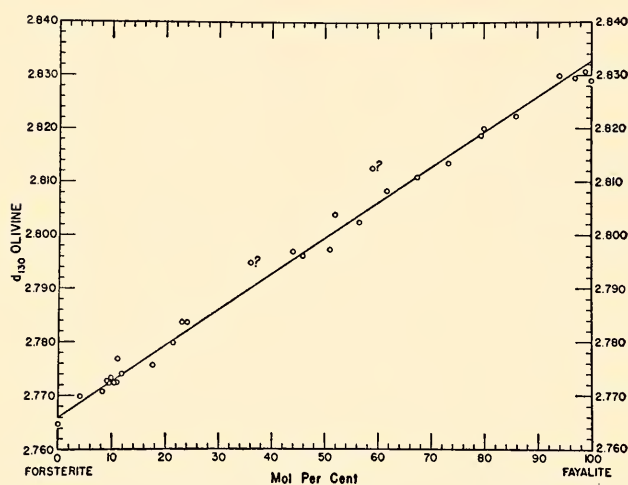


FIG. 11. Determinative curve for natural olivines of the forsterite-fayalite composition, using the 130 spacing.

standard of silicon. Most olivines have only minor amounts of calcium and manganese, so that the curve represents well the change of spacing for the pure system. Those olivines high in manganese, which are usually also very rich in iron, have spacings larger than those represented by the curve and hence can be easily detected. The value of  $d_{130}$  for synthetic tephroite, the manganese end member, is 2.8679. The spacings for synthetic forsterite and synthetic fayalite are 2.7647 and 2.8290, respectively; these points, however, were not used in fitting the straight line, which is based on analyzed natural and synthetic olivines. The line which best represents the natural olivine passes through the fictive end-member points 2.7660 and 2.8326 for forsterite and fayalite, respectively.

## GARNETS AND STAUROLITE

In the metamorphic rocks the almandite-pyrope garnets and staurolite serve as important indicators of the grade of metamorphism. It was found by Dr. L. Coes (Norton Company) that both could be made at 20,000 to 30,000 bars and 800° to 900° C, using various mixtures and fluxes, but their stability regions are not known. The geological occurrence of these minerals suggests that these extreme pressures—exceeding those thought to exist at the bottom of the deepest crustal downwarps—are not required for the formation of these minerals. Accordingly, experiments are under way at lower pressures and, in general, under more accurately defined conditions.

*Almandite* (Yoder). The exploratory runs indicate that the ferrous iron garnet almandite can be produced at a pressure at least as low as  $1000 \pm 5$  bars water vapor and at a temperature not exceeding  $810^\circ \pm 25^\circ$  C, and up to  $1030^\circ$  C at 10,000 bars, from glasses of the requisite composition as well as other starting materials. The color of the garnets varies from run to run, from cinnamon brown to black, and they are therefore believed to contain both ferrous and ferric iron. Above the stability limit of the garnet, fayalite + iron cordierite + hercynite, or their equivalent, are the products. Below 7000 bars the reaction rate diminishes rapidly. Some of the iron has obviously oxidized, and the products, although they may not represent equilibrium conditions, lie in a more complex system. These results point up our lack of knowledge of the behavior of iron in the presence of water vapor. This in itself is a difficult and fundamental problem. In addition, the relation of the garnet to the phase equilibria observed in the anhydrous system  $\text{FeO}-\text{Al}_2\text{O}_3-\text{SiO}_2$ , determined by Schairer and K. Yagi at atmospheric pressure, has yet to be found. In their study, the phases fayalite + iron cordierite + hercynite were obtained in compositions where almandite was expected. It appears

that the *laboratory* production of this garnet requires high pressures in order that the reaction may run at a high temperature where the reaction rate is appreciable yet within the field of stability of the garnet.

**Pyrope (Yoder).** Pyrope garnet has been produced by Dr. L. Coes at 900° C and 30,000 bars pressure only. All other conditions near those mentioned yielded a variety of products. In this Laboratory the pyrope composition yielded hydrous assemblages, the same as those found in earlier work at pressures up to 8000 bars. At a pressure of 10,000 bars enstatite was the primary product in metastable assemblages. It is evident that equilibrium has not been established in any of the extreme-pressure experiments on pyrope, and that much more work is required to outline the stability field of this garnet.

**Grossularite (Yoder).** An anhydrous grossularite ( $n=1.735$ ) was produced at 800° C and at water vapor pressures as low as 2000 bars from glass of the requisite composition held in sealed platinum tubes. Previous attempts at much lower pressures always yielded hydrogrossularite; some of the hydrogarnets, however, approached the anhydrous end member.

**Staurolite (Yoder).** Staurolite was not produced at pressures under 10,000 bars, starting with a variety of mixtures of the requisite composition. In its place the metastable assemblage almandite + quartz + corundum was obtained.

### ZEOLITES

**Analcite (Yoder).** It is often assumed in geological problems that the water pressure acting on a mineral at depth in the earth is equal to the force of the overlying rock. The laboratory experiments concerning mineral stability, where the water pressure is the total pressure on the system, are also run on this assumption. In fact, however, the water pressure may be slightly greater if the strength of the confining rock is taken into account, or may be less, depending on the accessibility of the

surface to the water. In many geological environments water has access to the surface by means of grain boundary diffusion or by flow through fractures. For this reason it is necessary to consider mineral stability under water pressures different from those of the total pressure acting on the system.

The mineral analcite,  $\text{NaAlSi}_2\text{O}_6 \cdot \text{H}_2\text{O}$ , was selected for the investigation of this problem because something was already

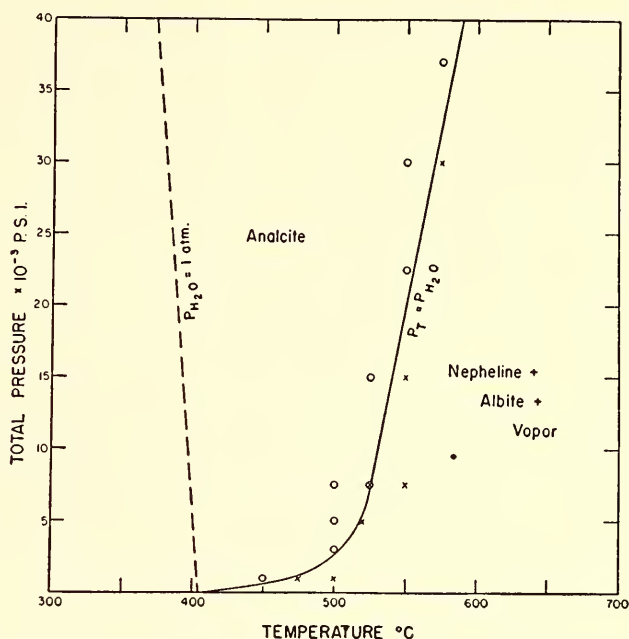


FIG. 12. Revised stability curve (solid line) of analcite when the water pressure is equal to the total pressure, and theoretical stability curve (dashed line) of analcite when the water pressure is one atmosphere.

known about its stability and because it grows quickly in its region of stability and decomposes readily outside that region. In figure 12 the upper limit of stability of analcite is given by the curve  $P_T = P_{\text{H}_2\text{O}}$ , where the total pressure is equal to the water pressure. The curve is based on new data, particularly in the lower pressure region. Only the critical runs are indicated. According to theory, the upper stability limit of analcite would be reduced to the dashed curve,  $P_{\text{H}_2\text{O}} = 1$  atm, if the water pressure were reduced to one atmosphere and the total pressure were allowed to vary. A family of such curves would exist for all water pressures between the limits of zero



and the total pressure. Experiments at 1000 psi and 5000 psi water pressure with pressures of argon sufficient to make the total pressure 30,000 psi yielded results duplicating the curve  $P_T = P_{H_2O}$ . The lowering of the equilibrium temperature was not observed. It is well known that analcite can be dehydrated without destroying its structure, and this behavior may have influenced the

results. Another possible, but improbable, explanation is that argon proxied for  $H_2O$  in the structure of analcite and, therefore, the total pressure was in effect 30,000 psi water pressure. It will be necessary to investigate a mineral which contains  $OH^{-1}$ , in which the water will not be readily subject to removal or replacement by inert gases.

## VAPOR TRANSPORT

*Solubility of solids in steam* (Morey). Study of the solubility of solids in superheated steam at high pressures is continuing to yield significant and surprising results. The basic concept of a solid's being dissolved by a gas sounds paradoxical, but its reality has been demonstrated with every substance tested. The actual values of solubility obtained in our experiments are always of a larger order of magnitude than would be predicted from simple theory. Every substance has its own vapor pressure, and elementary principles of physical chemistry would lead to the inference that the solubility of an oxide such as quartz or iron oxide in a gas should only be that resulting from the sum of the vapor pressure of the solid and the vapor pressure of the liquid. This is a wholly insignificant amount. A more rigorous treatment would take into account the fact that the high pressure of steam acting on the solid would increase its vapor pressure, an effect called the Poynting effect, the amount of which can be calculated by means of thermodynamic equations derived by Gibbs; but this correction is small, and the solubility values obtained experimentally are enormously greater than can be explained by conventional thermodynamics. For example, the vapor pressure of quartz at 600° C is not over one ten-thousandth of an atmosphere, but we have found the partial pressure of  $SiO_2$  in superheated steam at 600° C and 2000 atmospheres to be 15 atmospheres. To explain this phenomenon recourse must be had to statistical thermodynamics, and interaction must

be assumed between the constituents of the gas, water and silica, with probable formation of one or more compounds. This field of chemistry is so new that neither adequate theory nor the necessary data are available, but progress in this field will yield information directly applicable to the many geological processes in which solid material is transported by gas with resulting formation of ores and mineral deposits.

The experimental methods have been refined and the apparatus is yielding consistently satisfactory results. Two methods are in use, one dynamic, the other static. In the dynamic method, superheated steam at temperatures up to 600° C and at pressures up to 30,000 psi passes over the material being studied, contained in a metal bomb enclosed in an electric furnace. The condensed water is weighed and the amount of material dissolved in it is determined by analysis.

One of the materials studied was germanium oxide,  $GeO_2$ . This resembles silica in its general chemical behavior, and is even more soluble than  $SiO_2$ . At 15,000 psi and 400°, the solubility of  $SiO_2$  is 0.155 weight per cent, of  $GeO_2$  0.45; at 500°,  $SiO_2$  0.26,  $GeO_2$  0.86; and at 600°,  $SiO_2$  0.30,  $GeO_2$  1.24. The solubility at 600° C corresponds to a partial pressure of  $GeO_2$  in the gas of 19 atmospheres; the vapor pressure of  $GeO_2$  at this temperature is too small to measure. Other materials studied by this method were beryllium oxide, with gaseous solubilities at 15,000 psi of 66 parts per million at 400° C, and 120 parts per

million at 500° C; nickel oxide, with gaseous solubilities at 400°, 500°, and 600° of 22, 20, and 34 parts per million, respectively; and tantalum oxide, with solubilities at 400°, 500°, and 600° of 9, 30, and 13 parts per million, respectively.

A modification of this method uses superheated steam containing about 7 per cent carbon dioxide. The carbon dioxide has been found to affect the solubility. With quartz, it decreased the solubility 20 per cent; with nickel oxide, the increase in solubility was only a few per cent; with iron oxide, the increase was threefold;

with tin oxide, the increase was twenty-fold.

The dynamic method cannot be applied when the solubility of the solid in gas exceeds a few per cent, because of a tendency of the solid to separate out and clog the pressure lines. For such cases a static method, which is a refinement of the method used in the study of the system  $\text{H}_2\text{O}-\text{Na}_2\text{O}-\text{SiO}_2$ , has been developed. In some cases enormous solubilities have been found, in which the amount of dissolved salt is greater than the amount of the solvent water.

### ANHYDROUS SILICATE SYSTEMS

Studies of anhydrous silicate systems lead to results of both theoretical and practical value. New chemical compounds are discovered, their stability conditions are established, and their reactions with other components are noted. As part of the investigation, optical, crystallographic, and X-ray diffraction properties of the various compounds are measured. From data on the time necessary for the attainment of equilibrium in various compositions, qualitative information on rates of reaction and the viscosities of the liquid phase is obtained. Results of these studies are prerequisite to investigations of the same compositions with water as an additional component. In addition to their basic geological importance, studies of anhydrous systems may be expected to have practical application in glass, ceramics, slag metallurgy, and refractories.

*The systems  $\text{Na}_2\text{O}-\text{MgO}-\text{SiO}_2$  and  $\text{Na}_2\text{O}-\text{FeO}-\text{SiO}_2$*  (Schairer, Yoder, Keene). Very substantial progress has been made in our studies of the phase equilibrium relations in these two interesting systems. These fundamental ternary systems of rock-forming oxides are of extraordinary complexity, and the precise determination of the phase relations is a prerequisite to studies of more complex systems which may give an insight into the compositions of the alkaline pyroxenes and amphiboles.

Except in extremely siliceous compositions, the melts are not viscous, and equilibrium between crystals and liquid is attained in a few days or even hours. In both these systems there is a very extensive series of ternary compounds. Determination of the phase relations has been complicated by this large number of compounds, most of which melt incongruently to a liquid plus one or more different solid phases. Only with the aid of X-ray powder pattern techniques has it been possible to identify these many crystalline phases in intimate mixtures.

In the system  $\text{Na}_2\text{O}-\text{MgO}-\text{SiO}_2$ , 250 separate ternary compositions have been prepared and have been studied by the method of quenching. The preparation and study of about 50 additional compositions will be necessary before all the ternary invariant points are located accurately and the compositions of all the compounds determined. Last year (Year Book No. 52, fig. 11, p. 64) a preliminary diagram for this system was presented, showing the fields of primary crystallization of seven of the ternary compounds and the approximate temperatures of twenty ternary invariant points and one binary invariant point. No tie lines were shown because the compositions of some of these seven compounds were not then known with certainty. We present here as figure 13 a phase diagram showing the fields of sta-



bility and compositions of eight of the ternary compounds, tie lines, and precise values for twenty of the ternary invariant points and five binary invariant points. This phase diagram depicts the mutual melting relations of seven of the ternary

of equilibrium between crystals and liquid. Each of the new compounds, however, gives a very characteristic X-ray powder pattern by which it may readily be identified. As would be expected in a system consisting of only four different atoms—

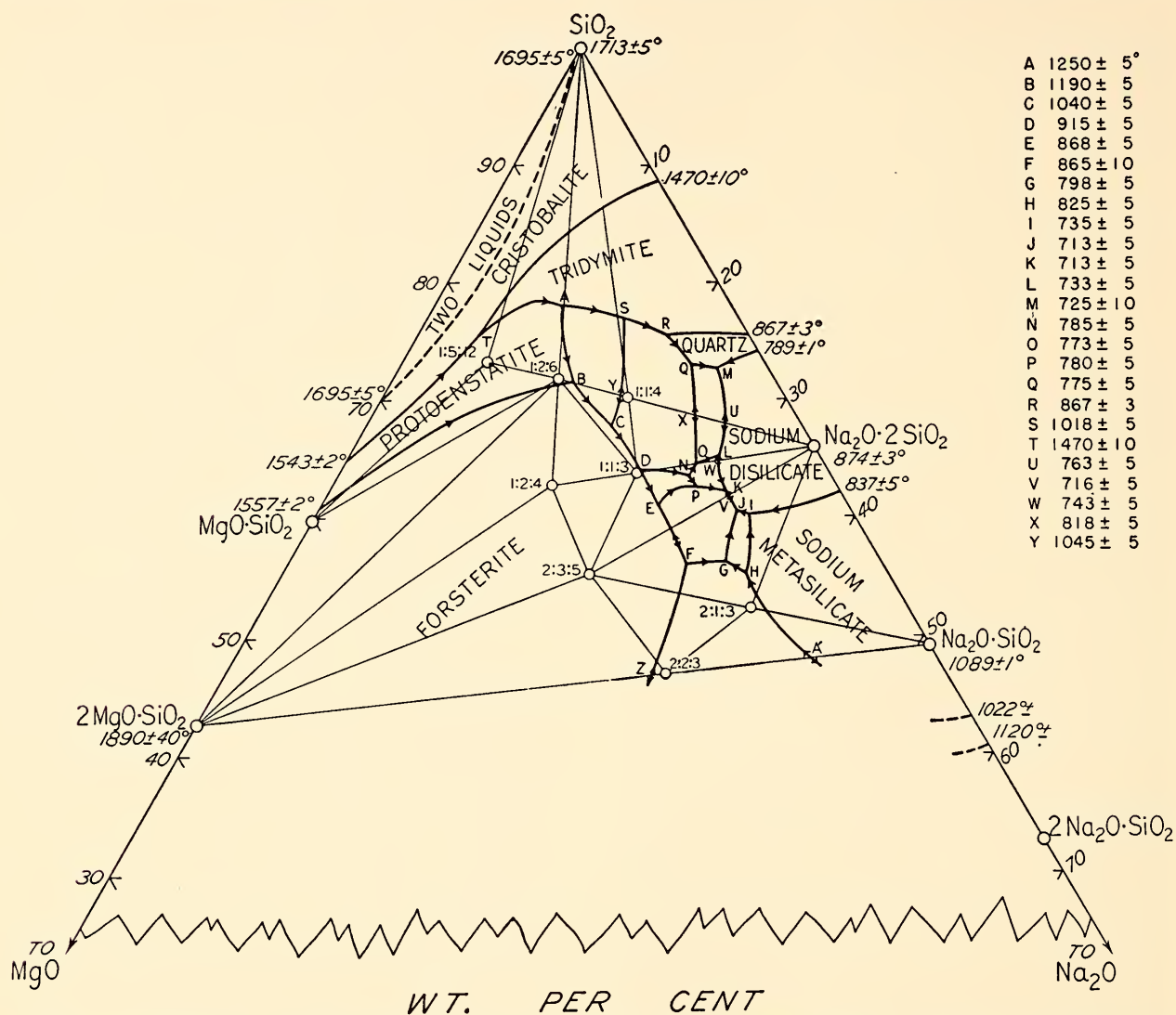


FIG. 13. Preliminary phase diagram for the system  $\text{Na}_2\text{O}-\text{MgO}-\text{SiO}_2$ , showing fields of primary phases, compositions of eight of the ternary compounds, tie lines, precise temperature values for twenty of the ternary invariant points and five of the binary invariant points, and the positions of two additional binary invariant points (equilateral triangle truncated at about 25 per cent  $\text{SiO}_2$ ). There is no tie line between  $\text{MgO} \cdot \text{SiO}_2$  and 1:2:6, and a tie line should be shown connecting  $2\text{MgO} \cdot \text{SiO}_2$  and 1:5:12.

compounds, with binary compounds from the limiting binary systems.

Several of the new compounds in the system  $\text{Na}_2\text{O}-\text{MgO}-\text{SiO}_2$  are so similar in optical properties that they are difficult to identify under the microscope, particularly when they are present as mixtures of tiny crystals suitable for the rapid attainment

sodium, magnesium, silicon, and oxygen—there are many coincident reflections from the many compounds and polymorphs. Each of these crystalline phases, however, has enough distinguishing reflections so that positive identification can be made even in mixtures. Some success has also been achieved in estimating the relative

proportions of the various crystals in mixtures, from the intensities of the reflections. The method is obviously qualitative, but aids considerably in the location of tie lines. The system  $\text{Na}_2\text{O—MgO—SiO}_2$  presents a real challenge to the X-ray crystallographer who aims to determine the multitude of spatial arrangements necessary for these compounds consisting of only four different kinds of atoms.

That portion of the ternary system below the two tie lines forsterite—2:2:3 and  $\text{Na}_2\text{O} \cdot \text{SiO}_2$ —2:2:3 is currently under intensive investigation. Additional ternary compounds appear in this portion of the system, and their compositions and mutual melting relations are now being studied.

Compositions with about 50 per cent or more silica can be prepared readily by simple synthesis from an appropriate dry crystallized sodium silicate, silica, and MgO. Difficulties are encountered, however, in the low-silica portion of the diagram. In such low-silica compositions part of the  $\text{Na}_2\text{O}$  is volatilized during the preparation of homogeneous melts, and the composition of the melt moves to give a melt of lower  $\text{Na}_2\text{O}$  content than was planned. The composition of each of these melts must be determined by a chemical analysis. These analyses are now in progress. Some of these low-silica melts are so fluid even below liquidus temperatures that it is not possible to quench the small samples in mercury rapidly enough to prevent formation of a rim of fine-grained quench crystals around idiomorphic primary crystals. This complicates X-ray determination of the crystalline phases.

In the system  $\text{Na}_2\text{O—FeO—SiO}_2$ , 68 separate compositions have been prepared and have been studied in equilibrium with iron crucibles in an inert atmosphere (purified nitrogen) by the method of quenching. Because of reaction between melts and the crucible, it is necessary to ascertain by a chemical analysis the final content of FeO and  $\text{Fe}_2\text{O}_3$  in each melt at the liquidus temperature for the particular composition. Except in compositions near the

FeO apex of the equilateral triangle used to represent ternary compositions, the  $\text{Fe}_2\text{O}_3$  content is low (less than 2.5 weight per cent). In figure 14 is presented a very preliminary phase diagram for the system  $\text{Na}_2\text{O—FeO—SiO}_2$ . Studies are now in progress on that portion of the diagram labeled "several fields." We know that this small area contains fields of stability of several ternary compounds, but the fields are so small that their precise location and the determination of the locations and temperatures of the many ternary invariant points is a matter of some difficulty.

The use of X-ray powder pattern techniques to determine the various compounds in the system  $\text{Na}_2\text{O—FeO—SiO}_2$  has been less rewarding than in the system  $\text{Na}_2\text{O—MgO—SiO}_2$ . The presence of iron results in considerable absorption of the copper radiation employed. This reduces the intensity of the reflection and also produces white radiation, thus increasing the background. Although there may be a slight advantage in shifting to radiation of longer wave length than copper, the inconvenience of changing X-ray tubes for samples containing only small amounts of iron is considerable. The determination of intensity will not, therefore, permit the detection of crystalline phases present in small amounts. One advantage of deciphering the system  $\text{Na}_2\text{O—MgO—SiO}_2$  first is that the iron analogues of the compounds identified will have similar X-ray patterns. This feature is aiding us greatly in fixing the composition of the compounds.

*The system  $\text{K}_2\text{O—MgO—Al}_2\text{O}_3\text{—SiO}_2$  (Schairer).* The join leucite—forsterite— $\text{K}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2$  was completed during the past year. The composition  $\text{K}_2\text{O} \cdot 6\text{MgO} \cdot \text{Al}_2\text{O}_3 \cdot 6\text{SiO}_2$  (the mica phlogopite is  $\text{K}_2\text{O} \cdot 6\text{MgO} \cdot \text{Al}_2\text{O}_3 \cdot 6\text{SiO}_2 \cdot 2\text{H}_2\text{O}$ ) lies in this join, as well as in the join leucite—forsterite—periclase, which was discussed last year (Year Book No. 52, p. 56). The system forsterite— $\text{K}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2$  is not binary, and cuts the primary phase volumes of forsterite, spinel, and orthorhom-



bic  $\text{K}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2$ . The join leucite—  
forsterite— $\text{K}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2$  cuts the pri-  
mary phase volumes of forsterite, leucite,  
spinel, and orthorhombic  $\text{K}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot$   
 $2\text{SiO}_2$ . Two piercing points of quaternary

leucite 40, forsterite 7,  $\text{K}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2$   
53 weight per cent.

A new join, forsterite— $\text{K}_2\text{O} \cdot \text{MgO} \cdot$   
 $3\text{SiO}_2$ — $\text{K}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2$ , has just been  
started, and the first five compositions are

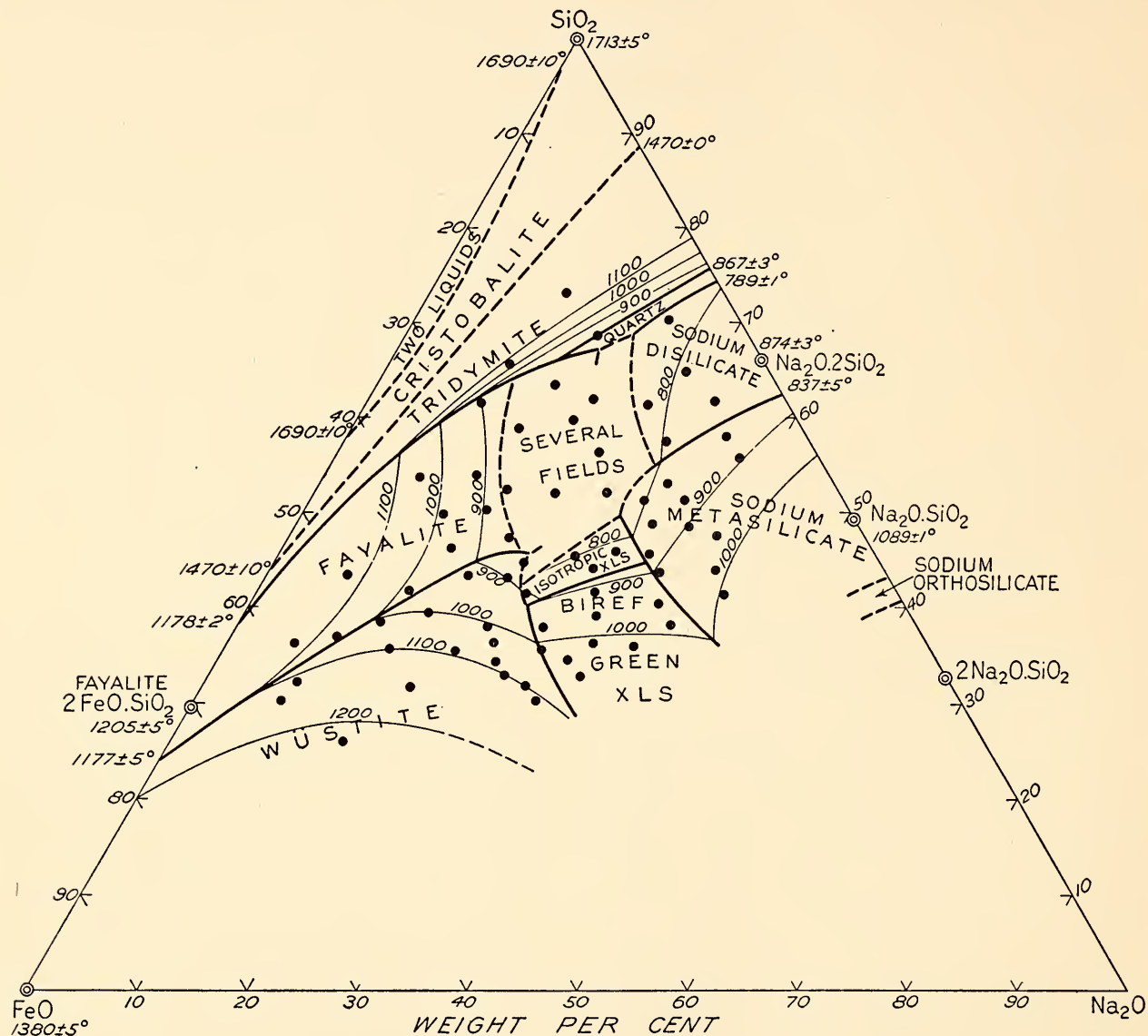


FIG. 14. Preliminary phase diagram for the system  $\text{Na}_2\text{O}$ — $\text{FeO}$ — $\text{SiO}_2$ , showing the broad relations between fayalite, wüstite, sodium metasilicate, sodium disilicate, the several forms of  $\text{SiO}_2$ , and some of the ternary compounds whose compositions and primary fields are now being studied. Black dots indicate the composition of melts previously studied, or now being studied. Isotherms indicate the broad temperature relations.

univariant lines lie in this join. The piercing point of forsterite + leucite + spinel + liquid lies at  $1470^\circ \pm 5^\circ$  C at the composition leucite 40, forsterite 20,  $\text{K}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2$  20 weight per cent; and the piercing point of leucite + orthorhombic  $\text{K}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2$  + spinel + liquid lies at  $1505^\circ \pm 5^\circ$  C at the composition

now in the course of preparation. This join should give a reconnaissance of a portion of the quaternary system on which we have very little information.

The binary system  $\text{K}_2\text{Si}_2\text{O}_5$ — $\text{Na}_2\text{Si}_2\text{O}_5$  (Kracek). In an earlier paper from this Laboratory, the phase equilibrium relations for the system  $\text{K}_2\text{SiO}_3$ — $\text{Na}_2\text{SiO}_3$ —

$\text{SiO}_2$  were described. The system has a simple binary eutectic join,  $\text{K}_2\text{SiO}_3$ — $\text{Na}_2\text{SiO}_3$ , and a rather complex binary join,  $\text{K}_2\text{Si}_2\text{O}_5$ — $\text{Na}_2\text{Si}_2\text{O}_5$ . The experimental data for the disilicate join included breaks in the liquidus curves for the two end components with a eutectic relation between them. It has now been shown that solid crystalline solutions play an extensive part in the equilibrium relations, with both of the components taking part in two limited solid-solution series at the liquidus, which, in place of the previously found eutectic relation, has a minimum at approximately 50 per cent across the join,  $734^\circ$ , with sodium-rich solid solution as the primary phase. There is a smoothly descending liquidus curve for the  $\text{K}_2\text{Si}_2\text{O}_5$ -rich solid solution, which joins the curve from the minimum at  $746^\circ$ , 40 per cent  $\text{Na}_2\text{Si}_2\text{O}_5$ . Toward  $\text{Na}_2\text{Si}_2\text{O}_5$  the curve from the minimum slowly rises to  $764^\circ$ , where it suffers a break at 76 per cent  $\text{Na}_2\text{Si}_2\text{O}_5$ , and then continues to rise to  $874^\circ$  for the pure sodium disilicate. Sub-solidus thermal-analysis studies, together with optical and X-ray data, prove the existence of an intermediate compound,  $\text{KNaSi}_2\text{O}_5$ , which can exist in two mutually slowly transforming forms. One of these is obtained in dry crystallizations, and has a rapid  $\alpha$ - $\beta$  transition at  $296^\circ$ ; the other, obtained by hydrothermal crystallization, has no rapid transition, but has much higher refractive properties than the first, and, accordingly, must be denser. Both of these principal forms of  $\text{KNaSi}_2\text{O}_5$  become unstable with respect to the solid-solution phase before reaching the liquidus. The hydrothermally crystallized form transforms at  $674^\circ$ , whereas the lower-index form obtained in dry crystallization apparently forms a continuous solid-solution series with the pure sodium disilicate, and hence has no definite decomposition point.  $\text{Na}_2\text{Si}_2\text{O}_5$  has several slowly convertible forms (see Year Book No. 52). In its high-temperature form A it can undergo two rapid transitions, one at  $707^\circ$ , the other at  $678^\circ$ . The transition at  $707^\circ$  is raised

to  $764^\circ$  by admixtures of  $\text{K}_2\text{Si}_2\text{O}_5$ , where the heat of transition contributes to cause a change in the slope of the liquidus curve. The  $678^\circ$  transition is rapidly lowered by the same admixtures, down to the  $296^\circ$  transition in the dry crystallized  $\text{KNaSi}_2\text{O}_5$  composition. In preparations still richer in K than  $\text{KNaSi}_2\text{O}_5$ , the transition quickly falls to  $290^\circ$  and remains at this value until it disappears at the composition  $3\text{K}_2\text{Si}_2\text{O}_5 \cdot 1\text{Na}_2\text{Si}_2\text{O}_5$ . This composition, or one near it, such as 4:1, should be a compound. Optical and X-ray studies of the 3:1 preparation, however, yield no indication of the presence of a compound, the X-ray pattern being much like that of potassium disilicate. On the other hand, thermal-analysis curves of compositions in this region yield arrests at  $470^\circ$ ,  $516^\circ$ , and  $746^\circ$ . The  $746^\circ$  arrest corresponds to the previously mentioned break in the liquidus curve, where the curve from the melting point of potassium disilicate meets the curve from the minimum on the liquidus. The  $516^\circ$  arrest is the result of the lowering of the  $594^\circ$  transition in  $\text{K}_2\text{Si}_2\text{O}_5$  by admixtures of sodium disilicate, and represents the lowest temperature at which the high-temperature modification of potassium-rich solid solutions can coexist with the sodium-rich solid solutions. The composition range of such coexistence is from about 9 to 40 per cent sodium disilicate. The  $470^\circ$  arrest extends in composition from about 7 to 40 per cent sodium disilicate and corresponds to no evident transformation other than that of the breaking up of a compound, such as the mentioned 3:1, into a heterogeneous mixture of potassium- and sodium-rich solid solutions. Further work is in progress, designed to obtain more detailed data in the region here discussed.

$\text{K}_2\text{Si}_2\text{O}_5$  has been known to have a transition at  $594^\circ$ . Recently there have been reported in the literature dilatometric data locating this transition at  $560^\circ$ , and another transition at  $250^\circ$ . We have re-determined these transitions, and have



found, as averages for the heating and cooling arrests, the temperature values of  $593^{\circ} \pm 3^{\circ}$  for the upper transition, and  $217^{\circ} \pm 4^{\circ}$  for the lower.

*The polymorphism of potassium-lithium silicates* (Kracek). There are reported in the literature compounds of  $K_2O$ ,  $Li_2O$ , and  $SiO_2$  having polymorphous modifications: (1) 1:2:6, in the order of the oxides given above, with transitions at  $490^{\circ}$  to  $500^{\circ}$ ,  $250^{\circ}$ , and  $40^{\circ}$  to  $50^{\circ}$ , measured dilato-

metrically. Thermal analysis yields two adjacent transitions (average of heating and cooling arrests) at  $520^{\circ} \pm 4^{\circ}$  and  $505^{\circ} \pm 5^{\circ}$ , and a lower transition at  $220^{\circ}$  on heating. No search has yet been made for the transition reported at  $40^{\circ}$  to  $50^{\circ}$ . (2) 2:5:7, a compound which is isotropic at room temperature, with a transition, measured dilatometrically, at  $390^{\circ}$ . Thermal analysis yields a transition at  $500^{\circ} \pm 4^{\circ}$  as average of heating and cooling arrests.

## STATISTICAL PETROGRAPHY

*Potash feldspar as a by-product of the chloritization of biotite* (Chayes). In most biotite granites, biotite is partially and sporadically transformed to chlorite. The transformation is particularly conspicuous in altered or weathered specimens, but even in the freshest material it is rarely entirely lacking. Potassium is an essential constituent in biotite, but chlorite analyses show only traces of it. The transformation, which is pseudomorphous and evidently involves little if any volume change, must thus release considerable quantities of potash. Despite the fact that the biotite-chlorite transformation is one of the earliest and best-known replacement reactions in petrography, the fate of this released potash seems to have attracted little attention.

In well stained thin sections of biotite granite, minute granules of potash feldspar are sometimes conspicuous along the cleavages of biotite or in chlorite which seems to have replaced that mineral. The amounts of chlorite and granular orthoclase are ordinarily so small as to make modal analysis quite impractical. In two specimens of a suite of fifteen from the Sierra de Guadarrama granite, Spain, sent to us by Dr. José Fuster, chlorite is unusually abundant, and in these two specimens granules of potash feldspar are far more frequently found in chlorite than in biotite. In the remainder of the specimens the tenor of chlorite and granular orthoclase is not significantly greater than in

many other granites of our collection. Although modal analysis is thus impractical, if not actually impossible, counting the flakes of biotite and chlorite with and without potash feldspar granules proved a fairly simple task. If the two chlorite-rich specimens are excluded, the final tally is:

|  | Potash feldspar granules |         |
|--|--------------------------|---------|
|  | Absent                   | Present |
| Fresh biotite .....                    | 2377                     | 74      |
| Chlorite and chloritized biotite ..... | 416                      | 431     |

The conclusion is so obvious that a statistical test of the result would be specious; in this particular granite, it is clear that the probability of occurrence of granules of potash feldspar is far greater in chlorite or chloritized biotite than in unaltered biotite, and it is natural to suppose that this feldspar is in some way a by-product of the biotite-chlorite transformation. Evidently not all the potash released by the breakdown of biotite is immediately fixed in feldspar, however, for the total amount of granular feldspar seems well below the stoichiometric equivalent, and nearly half of the chlorite flakes are free of it.

Petrographers have long argued that potash feldspar completes its crystallization very late in two-feldspar granites, and biotite is usually considered a rather "early" mineral. The evidence reviewed above implies that the stability field of

potash feldspar actually overlaps that of biotite, so that the feldspar continues to form at the expense of biotite. This is perhaps one of the strongest contrasts between the two- and one-feldspar granites, for the fabric of the one-feldspar granites suggests that in them the crystallization of alkali feldspar begins very early and does not long continue.

Granite, in general, contains little biotite and very little chlorite; the relation between biotite and chlorite is thus of rather minor consequence in the study of this rock. In the metamorphic rocks, on the other hand, the question of the stability relations between the pairs biotite-quartz and chlorite-orthoclase is of fundamental importance, for the appearance (or destruction) of biotite is one of the major markers in the grading of metamorphic rocks. The end stages of igneous crystallization thus provide a curious parallel with a common metamorphic reaction.

*Thin-section area and sampling variation in modal analysis* (Chayes). Most published studies utilizing modal analysis include no information about the extent of the measurements or the area over which each is spread. The extent of the measurement (the count length, in the case of point counting) controls the precision or reproducibility of the result. For a fixed grain size, the area of measurement determines the number of grain sections traversed in a single analysis, and the number of grain sections traversed in turn governs the sampling variance. The total analytical error of the method is the resultant of these two components. Evaluation and control of precision error for the point-counting procedure has been described in previous reports and in two Laboratory publications. Although satisfactory experimental studies of the precision of the continuous line integrators do not seem to have been made, the analogy with point counting is quite close, and it is reasonable to suppose that the line integrator as normally used yields results of

satisfactory precision for major constituents.

The second component of analytical error, sampling variation, is always more complex and, except in fine-grained rocks, much larger than the precision error. Our early concentration on finer-grained granites was prompted by a decision to avoid complications arising from sampling variation in the initial stages of the project. For some time it has been clear that greater tolerance in the matter of grain size would immensely broaden the scope of the work.

During the report year a study of this problem has been started. The first stage, an evaluation of the size of the effect, has been completed. This has been carried through on four rocks which differ so markedly in grain size that they can be graded without measurement. From a single hand specimen of each, 20 thin sections were cut. Each thin section contained a virtually perfect area of  $24 \times 40$  mm suitable for measurement. On half of the sections of each rock this area was subdivided into three parts,  $4$ ,  $8$ , and  $12 \times 40$  mm, respectively. Each such area, as well as the entire area of each of the undivided slides, was analyzed with a symmetrical grid of 660 points. Except possibly for slight disturbances arising from the fact that the symmetry of the grid differed for areas of different size, the precision error is thus the same for each suite of ten analyses, and differences in dispersion from suite to suite reflect the relative sampling efficiency of the different areas in each rock. The smallest area, a little less than  $\frac{1}{4}$  in<sup>2</sup>, is somewhat smaller than is ordinarily used for this type of work. The largest area, a little over  $1\frac{1}{2}$  in<sup>2</sup>, is much larger than the slides available to most petrographers, and is about as large as can be traversed in a single setting on standard mechanical stages.

Results for the fine- and coarse-grained specimens are shown in table 9 and figures 15 to 17. Table 9 lists mean values for major constituents. Figure 15 shows the individual values; the parallel lines are



3σ limits for the counting or precision error. It is evident that the precision component accounts for virtually all the analytical error in the fine-grained standard, and that at this grain size the largest measurement area is not notably more efficient than the smallest.

In the coarse-grained standard, on the other hand, results are much more widely dispersed at each area, and dispersion decreases sharply with increase in area. Even in the largest area, however, the spread of the results suggests that sampling variation

interpretation of results either impossible or misleading.

An example is shown in figure 17, a projection of the small- and large-area results for the coarse-grained standard in the mineralogical analogue of the "granite system" of experimental petrology. The slanted field extending upward into the diagram is the "thermal valley" in the dry system nepheline—kaliophilite—silica. Arrays of modal analyses of finer-grained granites ordinarily show one of two relations to this field: either all points lie

TABLE 9  
MEAN VALUES FOR MAJOR CONSTITUENTS OF FINE (WESTERLY) AND COARSE (CARNMENELLIS) GRANITES  
FOR DIFFERENT MEASUREMENT AREAS

| CONSTITUENT            | AMOUNT (VOL. PER CENT) FOR AREA |                     |                     |                     |
|------------------------|---------------------------------|---------------------|---------------------|---------------------|
|                        | 160 mm <sup>2</sup>             | 320 mm <sup>2</sup> | 480 mm <sup>2</sup> | 960 mm <sup>2</sup> |
| WESTERLY, RHODE ISLAND |                                 |                     |                     |                     |
| Quartz .....           | 26.5                            | 27.6                | 27.0                | 27.3                |
| Potash feldspar .....  | 37.7                            | 37.0                | 36.3                | 37.4                |
| Plagioclase .....      | 30.5                            | 30.4                | 31.4                | 30.5                |
| CARNMENELLIS, CORNWALL |                                 |                     |                     |                     |
| Quartz .....           | 31.8                            | 33.0                | 34.3                | 31.8                |
| Potash feldspar .....  | 28.9                            | 29.5                | 25.2                | 29.9                |
| Plagioclase .....      | 25.9                            | 24.6                | 26.6                | 25.8                |

overshadows the precision error. This relation is shown more clearly in figure 16, in which for each area the precision error is shown by an open circle, the observed standard deviation by a solid circle, and the 0.90 confidence band about the observed standard deviation by a vertical line. For the fine-grained standard the precision error in every case falls within the 0.90 confidence interval. The sampling variation component, if real at all, is trivial. In the coarse-grained standard, the precision error is always below, and usually far below, the lower bound of the 0.90 confidence interval; by far the larger contribution to the total analytical error comes from sampling variation, and this variation may often be so large as to make detailed

*within* the valley, or there is overlap *on one side* of the valley. Firm exceptions to this rule are so far unknown. Conformity to it is readily explained in magmatic terms, but cannot be rationalized by any of the nonmagmatic hypotheses now known. The distribution of analyses with regard to the thermal valley is thus a matter of prime importance. From the scatter of the small-area results (open circles) in figure 17 one might conclude either that this particular coarse-grained granite is a violent exception to the rule, or that coarse-grained granites in general differ from fine-grained ones. But the scatter of the large-area results (solid circles) is obviously much less; although two of the ten values do lie outside the valley

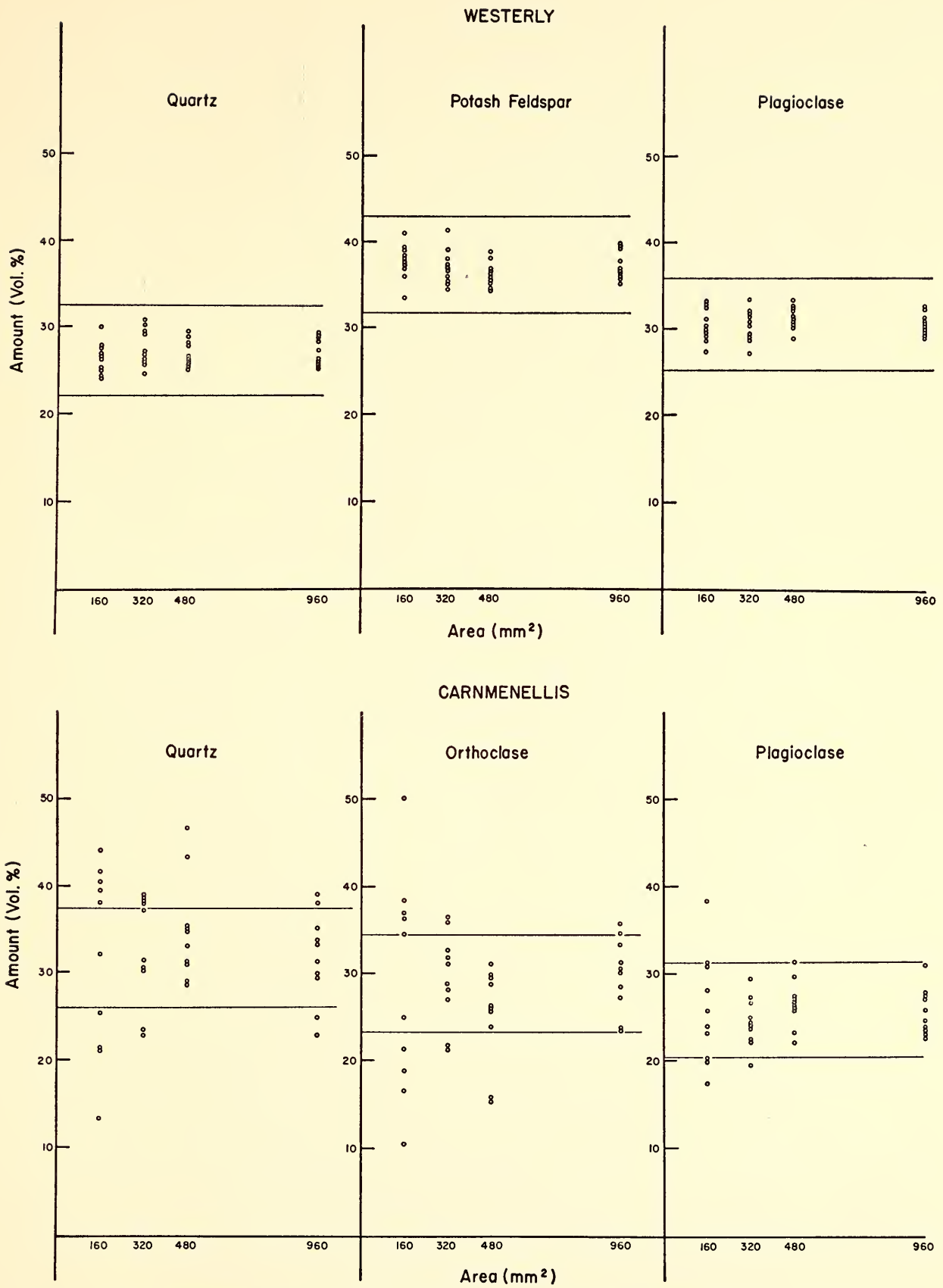


FIG. 15. Scatter diagrams for the fine (Westerly) and coarse (Carnmenellis) granite specimens



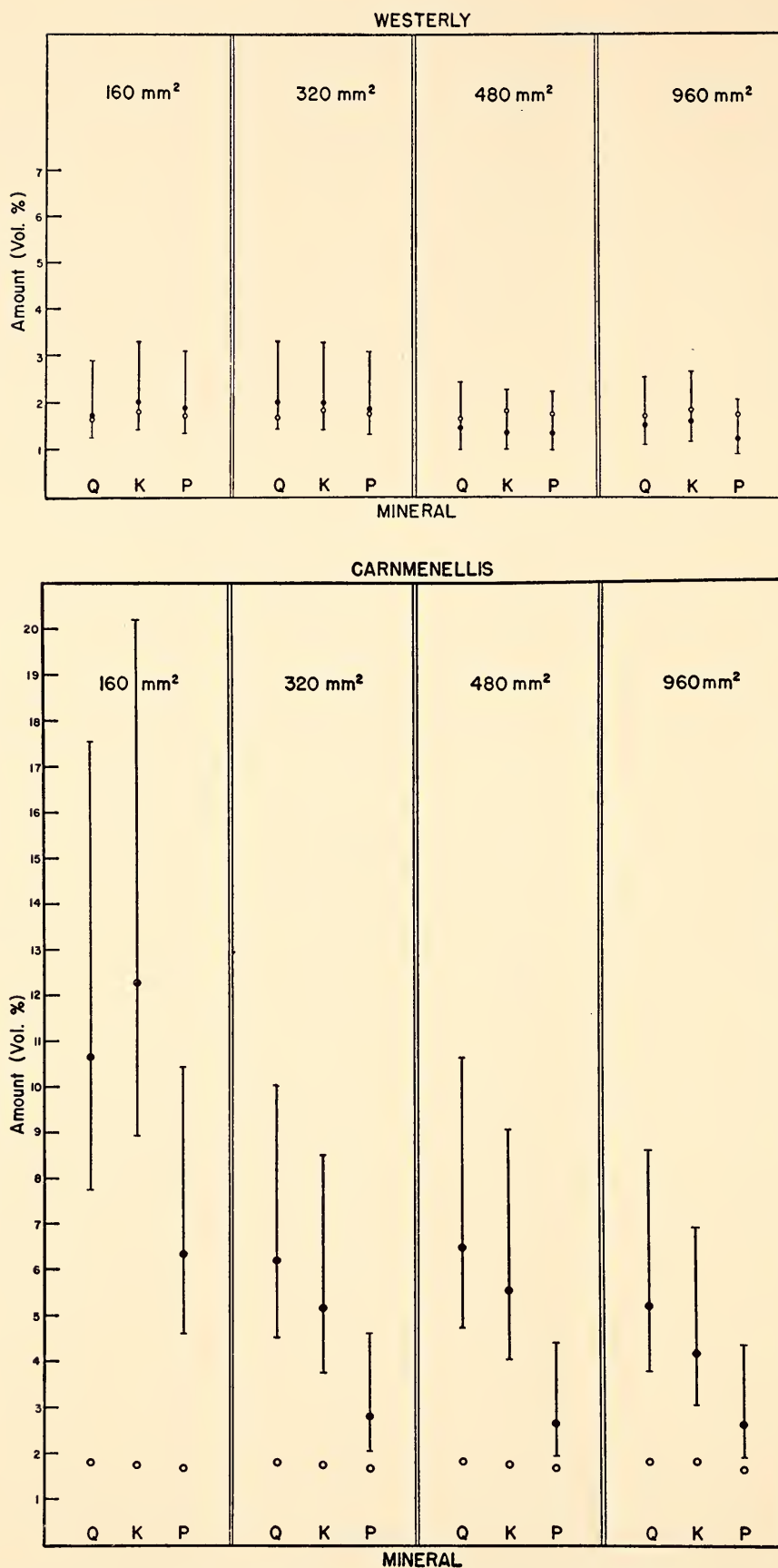


FIG. 16. Precision error (open circles), observed analytical error (solid circles), and 0.90 confidence interval about observed error (solid lines) for major constituents in the fine (Westerly) and coarse (Carnmenellis) standards. *Q*, quartz; *K*, potash feldspar; *P*, plagioclase.

on the Or side, both are distant from the border by less than the counting error. (The frequency of overlap on the quartz side is quite as pronounced for the large- as for the small-area results.)

Now all the results shown in the figure are for thin sections cut from the same

problem. In the present case, for instance, we immediately wish to know whether the dual overlap of the thermal valley is real or spurious.

The ten analyses of each suite may be formed into 45 pairs or duplicates. For the small-area results, 18 of these dupli-

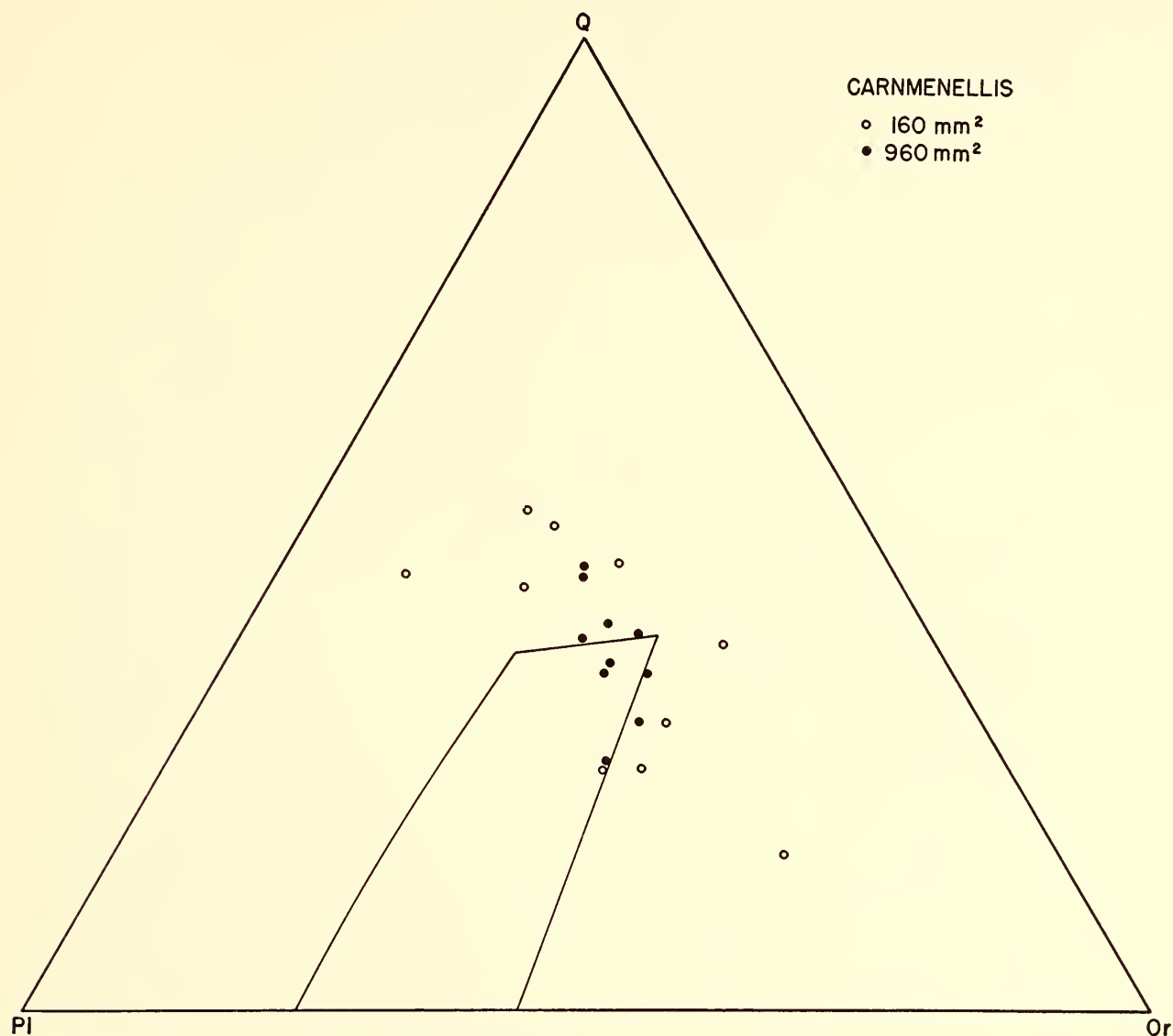


FIG. 17. Quartz (*Q*), plagioclase (*Pl*), and potash feldspar (*Or*) ratios for the small and large measurement areas of the coarse standard. Open circles, small areas; solid circles, large areas.

hand specimen, and all are subject to the same precision or counting error. The difference in scatter thus arises from the sampling variation component of the total analytical error. Even for the large-area results the scatter is enough larger than would be expected from counting error alone so that decisions about its reduction by replication should probably be considered only in relation to some specific

cates would yield averages above the quartz boundary of the valley, and this would be true also of 16 of the large-area pairs. But, whereas 14, or almost one-third, of the small-area pairs would yield averages to the right of the Or margin of the valley, only *two* of the large-area pairs would do so, and on the scale of the figure these two would actually graze the boundary.



In similar fashion, 120 sets of triplicates may be formed from each set of ten analyses. More than 20 of the small-area triplicates, but only *one* of the large-area triplicates, would preserve the Or overlap. The quartz overlap would be about the same for both sets of triplicates. The dual overlap is thus unaffected if two or three small areas are measured, but vanishes, for all practical purposes, if two or three large ones are used.

*Effect of choice of origin on mean and apparent dispersion of cyclical data* (Chayes). Cyclical data occur in virtually all branches of the natural and social sciences, and their treatment is the object of recurrent discussion. In geology the most important periodic data are the orientation measurements used in petrofabrics and structural work generally. The simplest example is the so-called two-dimensional fabric, in which all the orientation elements are measured in, or projected onto, some specific reference plane, such as the compass rose, the equatorial plane of a stereogram, the axial plane of a fold, and so on. In such a plane it is still necessary to decide on some point as origin. In geology the starting point is usually arbitrary, as when azimuths are recorded on a scale which increases clockwise from  $0^\circ$  at north, or declinations are recorded on a scale which increases from  $0^\circ$  at the horizon. There are occasional pleas for the use of origins which are "natural," "geologically significant," or intuitively more reasonable than the horizon or the north star.

For many years there has been a suspicion among structural geologists that a preferred orientation could be detected almost wherever one was sought, provided only that the search were sufficiently meticulous. Recently there has been a revival of skepticism on this score, and it is now pretty generally agreed that one must often test not only for the location, but also for the existence of low-intensity preferred orientations. A number of tests have been

proposed, each in one way or another less than satisfactory.

The most recent published suggestion is that the sample dispersion must be significantly less than the theoretical value calculated for an isotropic parent, and for this purpose the "rectangular" or equal-frequency distribution is taken as the model of isotropism. As originally announced, the test called for calculation of the sample statistics from an "arbitrary" or "unique" origin. It was soon pointed out that the dispersion would vary with the choice of origin, but no general relation between origin and variance was offered.

This relation is not difficult to deduce, and permits the location of the origins (and means) yielding minimum or least variance before calculation of the variances. Specifically, if  $R$  is the range (a full period),  $O$  is the first origin,  $A$  the second,  $n$  the total number of observations,  $a$  the number lying between the two origins,  $k$  the fraction  $a/n$ ,  $\bar{x}_a$  the mean of the points lying between the two origins, and  $\bar{x}_1$  the mean of the full array, both means being calculated from  $O$ , then the difference between variances calculated from the two origins is

$$2R \frac{n}{n-1} \left\{ (\bar{x}_a - \bar{x}_1) + \frac{R}{2} (1 - k_a) \right\},$$

a quantity which is easily computed.

The quantity

$$I_v = \frac{n}{n-1} \left\{ (\bar{x}_a - \bar{x}_1) + \frac{R}{2} (1 - k_a) \right\}$$

may be used as a convenient indicator of variance differences. A series of  $I_v$  values calculated from any starting point to new origins systematically distributed through one full period may be used to find the mean from which the variance is largest, the mean from which it is smallest, and the maximum variance difference which may be generated by choice of origin. The origin which will yield the smallest variance, for instance, is the one for which  $I_v$  takes its largest negative value, and the mean calculated from this point is  $\bar{x}_a = \bar{x}_1 + kR$ .

Application of these results to the proposed test of departures from isotropism is obvious. If the announced dispersion happens to be significantly small, it does not matter how the origin from which it was calculated was selected. But failure to establish anisotropism is essentially meaningless unless the origin used is one for which the variance is least. In this case, however, the test is critical with regard to both alternatives, for if it fails from such an origin, it will also fail from any other origin, and the conclusion must be that the sample offers no evidence of departure from isotropism in the parent population.

*Composition of feldspars in two-feldspar granites* (Robbins, Chayes). Synthetic studies of feldspar ordinarily deal with pure end members or binary mixtures. The feldspars found in common rocks are rarely if ever pure end members. In laboratory work, for instance, the symbol "Ab" denotes a material of pure albite composition, but to the mineralogist both the symbol "Ab" and the word "albite" include by definition plagioclase compositions carrying as much as 10 per cent of anorthite. Similarly, "Or" denotes the pure end member,  $\text{KAlSi}_3\text{O}_8$ , to the laboratory worker, although natural materials entirely free of soda are exceedingly rare.

Optical procedures for determining the An (or lime) content of natural plagioclase are, of course, well known, but they are often tedious and time-consuming. No optical method for determining the Ab (or soda) content of natural potash feldspars is known. Modal analyses ordinarily report a total plagioclase value without reference to An content, and a total potash feldspar value without reference to Ab content. Proper integration of field and laboratory findings therefore requires information of both types, and a program aimed at providing this information for a sizable suite of granite specimens has been in progress for two years.

Sample preparation and the X-ray procedures used for plagioclase determination were described in Year Book No. 52 (pp.

46-47). The alkali feldspars were first inverted to the monoclinic form, either by prolonged heating at  $1040^\circ\text{C}$  or by hydrothermal recrystallization in sealed platinum tubes at  $800^\circ\text{C}$  and 15,000 psi of water vapor. The angular distance between the  $\bar{2}01$  spacing of the unknown and that of an internal standard (Amelia albite with  $\bar{2}01$  at  $22.06^\circ \pm 0.01$ ) was used as a measure of composition, in the way first proposed by Bowen and Tuttle. We have redetermined the Bowen-Tuttle curve with glasses spaced at 5 per cent intervals between  $\text{Or}_{100}$  and  $\text{Or}_{60}$ . In this range the regression of composition on spacing is  $Y = 113.63 - 103.23X$ , where  $X$  is the angular distance in degrees and  $Y$  is the composition in parts per hundred of Or (weight per cent). This result is in good agreement with the original.

The basic assumption—that the lattice parameters of a sanidinized natural microcline are identical with those of a synthetic feldspar of the same composition—is perhaps open to some question. Furthermore, our specimens almost certainly depart from ideal composition with regard to trace elements, and may also contain a little lime. Three flame photometer analyses made for us by W. W. Brannock of the U. S. Geological Survey nevertheless indicate that the net effect of these difficulties may be rather small. In table 10 the X-ray and flame photometer estimates of Or are compared. The first flame photometer entry is simply the potash reported by Brannock multiplied by the appropriate gravimetric constant. The second is this result adjusted for nonpotassic impurities (quartz and plagioclase) estimated by fragment count. Agreement as good as that indicated in the table would certainly be ample for practical work. Considerable further chemical checking will be required in order to discover whether such agreement can be expected in routine work. We are reasonably confident, however, that the X-ray results are at least internally consistent, and that differences between



X-ray and chemical values, whether random or biased, will in general be small.

Specimens forming the basis of this report were selected from two-feldspar granites of our collection, for which adequate modes are available and in which perthitic albite is never more than a very minor accessory. Most of the material meeting these requirements is from New England, the southeastern United States, and Texas.

Our principal interest was in the mean values for Ab in plagioclase and Or in microcline, and in differences among these means from mass to mass. We were also

TABLE 10  
COMPARISON OF X-RAY AND FLAME PHOTOMETER ESTIMATES OF OR CONTENT OF MICROCLINE

| SPECIMEN                        | OR CONTENT |                  |      |
|---------------------------------|------------|------------------|------|
|                                 | X-ray      | Flame photometer |      |
|                                 |            | A                | B    |
| 41A9 (Bellingham, S. D.) . . .  | 82.2       | 80.7             | 81.9 |
| 17A3 (Milford, N. H.) . . . . . | 86.6       | 86.5             | 87.8 |
| 39A3 (Fitzwilliam, N. H.) . .   | 91.2       | 88.9             | 89.8 |

X-ray data by Robbins; flame photometer determinations by W. W. Brannock.  
 $A, Or = 5.917 (K_2O)$ ;  $B, Or = A/(100 - Q - Pl)$ .

concerned with the amount of variability which might be expected within masses; the literature offering no reliable guide in this connection, we felt obliged to sacrifice information about means in order to estimate variability by a fairly detailed study of a single mass. The final array of samples and the mean values are shown in table 11.

The variance of plagioclase values for all but the Fitzwilliam group is partitioned as follows:

| Source                          | Degrees of freedom | Mean square |
|---------------------------------|--------------------|-------------|
| Between granite masses . . . .  | 16                 | 95.32**     |
| Within granite masses . . . . . | 15                 | 5.89        |

Differences in content of plagioclase from mass to mass are so marked that they can easily be detected even if the average value for each mass is based on only two speci-

mens. Application of variance analysis assumes that the subsample variances which contribute to the residual mean square (in this case the "Within granite masses" entry above) are homogeneous, but actually we have no direct evidence to this effect. The large group (14 specimens of Fitzwilliam granite) was run to provide

TABLE 11  
AVERAGE AB IN PLAGIOCLASE AND OR IN MICROCLINE IN A SUITE OF GRANITE SPECIMENS

| Granite mass                | No. of specimens | Ab in plagioclase | Or in microcline |
|-----------------------------|------------------|-------------------|------------------|
| Fitzwilliam, N. H. . . . .  | 14               | 76.7              | 90.0             |
| Barre, Vt. . . . .          | 2                | 88.6              | 83.3             |
| Woodbury, Vt. . . . .       | 2                | 80.0              | 89.4             |
| Bradford, R. I. . . . .     | 2                | 78.7              | 88.9             |
| Westerly, R. I. . . . .     | 2                | 77.0              | 87.6             |
| Concord, N. H. . . . .      | 2                | 87.9              | 89.0             |
| Milford, N. H. . . . .      | 2                | 73.9              | 86.6             |
| North Jay, Me. . . . .      | 1                | 82.6              | 86.0             |
| Clark Island, Me. . . . .   | 2                | 76.9              | 88.6             |
| North Sullivan, Me. . . . . | 2                | 72.8              | 84.3             |
| Pownal, Me. . . . .         | 2                | 78.6              | 89.4             |
| Elberton, Ga. . . . .       | 2                | 76.2              | 91.0             |
| Mt. Airy, N. C. . . . .     | 2                | 81.4              | 90.4             |
| Salisbury, N. C. . . . .    | 2                | 98.4              | 90.2             |
| Winnsboro, S. C. . . . .    | 1                | 73.6              | 88.2             |
| Blairs, S. C. . . . .       | 2                | 79.2              | 92.8             |
| Fredericksburg, Tex. . . .  | 2                | 92.2              | 88.9             |
| Llano, Tex. . . . .         | 2                | 77.7              | 91.7             |

Measurements by Robbins.

information on this score, and the result, although not in any sense a demonstration of homogeneity, does indicate that the use of such a large control group was something of an extravagance. The observed standard deviation for the 14 Fitzwilliam specimens is 2.64; the sample standard deviation for combined within-mass variability and experimental error is the square root of the "Within granite masses" entry above, 2.43.

Similarly, the variance of Or content of potash feldspar, excluding the Fitzwilliam group, is partitioned as follows:

| Source                          | Degrees of freedom | Mean square |
|---------------------------------|--------------------|-------------|
| Between granite masses . . . .  | 16                 | 11.31       |
| Within granite masses . . . . . | 15                 | 8.26        |

In this case the between-mass mean square fails of significance, and a glance at table 11 shows why. Although 16 of the 18 mean values for Ab content of plagioclase are in the oligoclase range, even these are spread over an interval of 17 per cent, and the two albite-bearing masses enlarge this interval to 27 per cent. In contrast, all 18 of the Or values for microcline fall in the interval 83 to 93. Evidently the range of composition of microcline is very much smaller than that of plagioclase in two-feldspar granites. The within-mass standard deviation obtained is 2.87, which again compares favorably with the value 2.45 yielded by the Or values of the Fitzwilliam suite. To the extent that the Fitzwilliam mass is typical of rocks of this type, there is a clear indication that the same information about within-mass variation, and better information about differences between masses, would have been obtained by a symmetrical arrangement of the observations in sets of three.

For the sharply restricted range of composition of potash feldspar in granites of this type we have at present no explanation. All the rocks involved are biotite or biotite-muscovite granites, in all of them the potash feldspar displays the anhedral to interstitial habit characteristic of it in this group, and in all of them plagioclase is a major constituent. In other respects, however, they are highly variable. They may be either rich or poor in biotite. Muscovite may be either abundant or virtually lacking. Average quartz content may run as low as 24 per cent or as high as 33 per cent. In some, potash feldspar is considerably more abundant than plagioclase; in others, plagioclase is strongly dominant over potash feldspar. The habit of the potash feldspar strongly suggests that it forms very late; perhaps in fixing its composition the physical environment of the final stages of consolidation—which must be much the same from rock to rock—exerts more influence than bulk composition.

*Modal analysis of perthite in the Bullfrog granite, Mount Baxter quadrangle,*

*California* (Moore, Chayes). In this granite, one of the major members of the Sierra Nevada complex exposed in the Mount Baxter quadrangle, the alkali feldspar is conspicuously and rather coarsely microperthitic, despite the fact that the over-all modal composition of the rock (see table 12) is of a type in which perthite is not usually of much quantitative importance. The habit of the alkali feldspar in this rock is also somewhat anomalous with regard to its modal composition. Instead of showing the anhedral to interstitial habit so common in microcline of the two-

TABLE 12

MODE OF THE BULLFROG GRANITE  
(Volume per cent, mean of 8 thin sections)

| Component                     | Per cent |
|-------------------------------|----------|
| Quartz .....                  | 25.0     |
| Alkali feldspar .....         | 42.0     |
| Plagioclase .....             | 30.4     |
| Biotite .....                 | 1.1      |
| Hornblende .....              | 0.4      |
| Chlorite .....                | 0.2      |
| Opaque accessories .....      | 0.5      |
| Transparent accessories ..... | 0.3      |

Analyses by Moore.

feldspar granites, it is for the most part euhedral or markedly subhedral, like the alkali feldspar of the one-feldspar granites. (Similar though less extreme anomalies in the composition and habit of alkali feldspar have been noted in other hornblende-bearing, two-feldspar granites.) Contacts of feldspar grains with one another are crenulate and irregular, but the interlocking is confined to the outermost margins of the crystals, and wherever alkali feldspar grains abut against quartz the contact is consistently sharp and straight. Small grains and irregular veins of albite occur along feldspar-feldspar contacts and also within single crystals of alkali feldspar, in which they lie along the Carlsbad composition face. There is often a distinctive thinning of perthitic albite seams toward the margins of the alkali feldspar crystals, so that the central por-



tions appear somewhat richer in perthite than the outer regions.

Because of the habit of the alkali feldspar crystals and the coarseness of the perthitic albite in them, it has been possible to estimate their modal composition by point count. We are not aware that this type of measurement has been successfully performed on other rocks of similar grain size. The results indicate, clearly if rather indirectly, that perthitic albite is in fact somewhat less abundant in the marginal than in the central portions of the alkali feldspar grains. Analyses were made on large, carefully stained thin sections. In eight thin sections a total of 67 of the largest alkali feldspar grains were analyzed, with intercept distances of 0.05 mm along and 0.5 mm between traverses. The average count length was 390 per crystal, so that the reproducibility is rather poor; the main objective of the work, however, was an estimate of the average value for each slide, and the over-all precision is satisfactory for this purpose. Figure 18 is a scatter diagram of the results. Differences between specimen means are certainly small. With the information at our disposal they cannot be shown to be significant, although the field distance between sample sites may be as much as seven miles.

The only evidence of "zoning" in the alkali feldspar crystals is the apparent depletion of perthitic albite toward the margins, so that it is difficult if not impossible to separate "cores" from "mantles" in a fashion which will permit an objective test of this depletion. If the crystals were all of the same size, or were drawn from a single population of sizes, however, it would be true, in general, that the smaller cross-sectional areas exposed in thin section would be drawn from outer portions of the crystals, and the larger cross-sectional areas would necessarily penetrate deeper into the crystals. If the outer portions were consistently poorer in perthitic albite, one would expect to find that smaller grain sections would usually contain less per-

thitic albite than larger ones. The observed perthitic albite content thus ought to vary directly with the section area as indicated by the total count length, and this does seem to be the case, although an exact test is difficult for a number of reasons.

The problem has been the subject of considerable discussion among members of the Earth Science Panel of the American Statistical Association. The general ap-

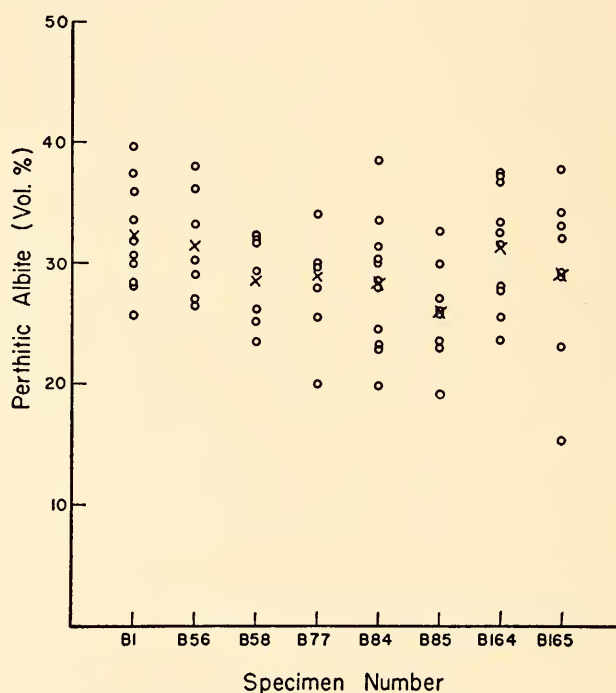


FIG. 18. Perthitic albite (volume per cent) in alkali feldspar grains in eight specimens of the Bullfrog granite. Open circles, individual grains; crosses, specimen means. (Analyses by Moore.)

proach taken may be briefly indicated by two comparisons: (1) in seven of the eight specimens, the smallest alkali feldspar grain contains less perthitic albite than the largest; and (2) if the grains are arranged in order of increasing size and the cumulative counts tallied, in all eight specimens the lower third of the cumulative count indicates a smaller perthitic albite content than the upper third. The full data and proposed solutions will be presented elsewhere.

It seems reasonable to suggest that all the visible perthitic albite has exsolved from an initially homogeneous alkali feldspar, and that from the outer portions of the crystals

this exsolved material has diffused into the matrix, where it forms seams and stringers of fine-grained albite, sometimes mantling

the potash feldspar crystals, and readily distinguishable from the primary plagioclase of the rock.

### MISCELLANEOUS STUDIES

*Elastic properties of minerals* (Yoder, Weir). In Birch's study of the constitution of the earth's interior, he concluded that at between 200 and 900 km depth the rate of rise of seismic velocity is too great for a homogeneous layer, and that a gradual change of composition, or phase, or both, is indicated. He suggests that there is a gradual shift toward high-pressure modifications of the ferromagnesian silicates, and these are probably close-packed oxides. The elastic ratio, which is directly related to the seismic velocities, for periclase, corundum, and rutile has been found to satisfy some of the postulated parameters at the depths in question. Spinel is a very common mineral belonging to the same general class of materials, and, since its density and composition were in accord with those required, it was considered desirable to obtain its elastic ratio. The elastic ratio is simply the inverse of the product of the adiabatic compressibility and the density. This may be approximated, then, by measuring the isothermal compressibility and the density. The preliminary value of the compressibility up to 10,000 bars was found to be  $4.1 \times 10^{-7}$  for natural spinel (U. S. Nat. Mus. 104569) from Ceylon. Its density was found to be 3.58, and hence the approximate elastic ratio is  $68 \text{ (km/sec)}^2$ . This value is well within the estimate of the parameter at the depths considered by Birch. This constitutes additional support for the idea that the possible new phases at depth have structures similar to those of the oxides.

*The Yellowstone rhyolite plateau* (Boyd). A field investigation in Yellowstone Park, begun in 1951, is now being brought to a conclusion. This investigation has had two principal aims: first, to see what light could be shed on the mechanism of eruption of welded tuffs; and second, to work

out the volcanic history of the rhyolite plateau.

Welded tuffs are one of the commonest types of silicic volcanic rock. Their distinguishing characteristic is that, although they have the textures of fragmental volcanics, they have been indurated by welding to a point where, in hand specimen, they simulate lavas. The mechanism of eruption of welded tuffs is a problem, inasmuch as no observed volcanic eruption has ever produced a deposit with their textural and structural features. The field evidence in Yellowstone supports the theory that they have been emplaced as hot avalanches of fragmented viscous lava.

A thermodynamic analysis of the eruptive process suggested by field evidence has been undertaken. The temperature interval between the original magma temperature and the welding temperature of the erupted tuff will have a maximum value of about  $100^\circ \text{C}$ ; in the average case it will be somewhat less. This estimate is based in part on the data of Tuttle and Bowen (Year Book No. 52, p. 50) and in part on an experimental determination of the minimum temperature at which a rhyolitic tuff will weld.

The amount of heat lost during emplacement of a tuff avalanche will depend primarily on the thickness of the tuff flow, the manner in which the flow moves, and the time elapsed during emplacement. Reasonable values for all these quantities can be found either from field evidence or from the data available on nuées ardentes. Assuming a collapsed thickness of 30 meters, an average time of emplacement of one hour, and turbulent flow in the moving avalanche, the loss of heat during emplacement will reduce the temperature by less than  $10^\circ \text{C}$ —a value well within the estimated limits.



Various heat effects are associated with the eruption of a magma while the eruption is contained within the conduit. By far the most important of these effects can be the work done by the gas in moving the magma to the surface. Evidence in the case of welded tuffs, however, suggests that vesiculation may be delayed until the magma reaches a point very near or at the surface. Under these circumstances, the expanding gas bubbles rupture before their internal pressure rises above a few tens of bars. The work done, and hence the heat lost, will under these conditions be small.

An uncertainty involved in the analysis outlined above has been due to the possible effect of water dissolved in the glassy fraction on the welding temperature of a tuff. The presence of water in solution in the glass would be expected to reduce the welding temperature.

Analysis of a number of glassy Yellowstone welded tuffs reveals that they characteristically contain 2 to 3 per cent  $H_2O$ . This water can be interpreted as meteoric, but doubt has been raised by the fact that flow glasses of the same silicate composition and exposed to the same weathering conditions generally contain less than 0.1 per cent  $H_2O$ .

An answer to the problem has been suggested by some experiments, recently carried out, on hydration and dehydration of rhyolite glasses. The solution of water in a silicate melt produces considerable structural change. If the melt is quenched to a glass and the water is driven off at relatively low temperatures, the viscosity of the glass prevents structural readjustment, i.e. the glass has a "memory." This memory permits a dehydrated glass to be rehydrated readily in a bomb at temperatures as low as 300° to 400° C and vapor pressures as low as 25 to 50 bars. Originally anhydrous flow glasses subjected to the same pressure-temperature conditions fail to pick up any water at all. It was found that the memory in an originally hydrous glass could be destroyed only by annealing at 1500° C for several days. The rate at

which water can be driven off from hydrous glasses makes it appear relatively certain that any water remaining in the glassy fraction of a welded tuff after vesiculation will be lost by diffusion during emplacement. The results of these experiments indicate that the water now present in these glasses is probably of meteoric origin.

Reconnaissance and detailed mapping in the Yellowstone rhyolite plateau have revealed that its eruption took place in three principal stages. The oldest and youngest of these stages are marked by the eruption of flows, the intermediate stage by the eruption of welded tuffs. The youngest of the flows are extremely fresh in appearance and are among the largest ever found; individual flows cover areas on the order of 100 square miles and are up to 1000 feet thick. Eruption of the various units took place from vents scattered throughout the Park; no major volcanoes were built during the construction of the plateau.

*Norelco powder X-ray diffraction specimen changer* (Van den Heurk). The powder X-ray diffraction technique has become one of the most useful methods for the identification of minerals. It is now being employed in conjunction with the microscope in routine examination of quench products obtained in phase equilibrium studies. The precision of the technique also permits determination of the composition of minerals of certain types, such as the feldspars, pyroxenes, olivines, and micas. It is a tool used by practically every staff member of the Laboratory, and hence time on the two available units is at a premium. In order to make the most use of the Norelco Geiger-counter spectrometers, an automatic specimen changer was constructed which permits 24-hour operation of the apparatus without attendance. The device consists of a turntable on which 12 specimens may be clamped in a plane flush with the customary specimen holder. The turntable is

rotated one position by a motor, governed by suitable microswitches, at the end of each record. While the turntable is revolving, a solenoid simultaneously disengages the driving clutch and engages a magnetic reversing clutch. The latter clutch is connected to a geared motor which drives the scanning arm of the

spectrometer back rapidly to its starting position, fixed by adjustable stops. In turn, another microswitch operates the solenoid, releasing the double clutch, and a new record is begun. The device may be installed with little alteration of the basic unit and operates without loss of accuracy and precision.

## MISCELLANEOUS ADMINISTRATION

*Student investigators.* One innovation this year has been a co-operative program with the Geology Department of Johns Hopkins University. Three men, C. A. Hopson, V. J. Hurst, and J. G. Moore, have worked part time at this Laboratory. They were able to acquire experience in our techniques and experimental methods while studying facets of their thesis problems which were of interest both to them and to this Laboratory. It is particularly desirable that staff members be exposed to enthusiastic, knowledge-seeking youth. It is hoped that the co-operation will continue and expand.

*Petrologists' Club.* The Laboratory was host to the Petrologists' Club during its 1953-1954 season. Six sessions were held, attended by as many as one hundred scientists. The programs consisted mainly of papers concerning latest developments in studies of the major mineral groups. When possible, the views of both the field geologists and the laboratory investigators were presented. Discussion following the papers was spirited and informative.

The following papers were presented:

"Review of feldspar problems," by O. F. Tuttle (Pennsylvania State University), F. Laves and J. R. Goldsmith (University of Chicago), and W. S. MacKenzie (Geophysical Laboratory).

"Review of pyroxene problems," by H. H. Hess (Princeton University) and J. F. Schairer (Geophysical Laboratory).

"Orijärvi region, Finland," by H. V. Tuominen (Lehigh University).

"Experimental studies of the humites," by A. Van Valkenburg (National Bureau of Standards).

"Experimental studies in the amphibole group," by F. R. Boyd, Jr. (Geophysical Laboratory).

"Effect of trace elements on crystal growth," by S. Zerkow (Naval Research Laboratory).

"Distribution of minor elements: theoretical and experimental," by H. P. Eugster (Geophysical Laboratory).

"Review of clay mineral problems," by S. B. Hendricks (Department of Agriculture), C. S. Ross (U. S. Geological Survey), and W. F. Bradley (Illinois Geological Survey).

*Seminars.* In addition to the Petrologists' Club, the Laboratory has conducted a weekly series of seminars, with papers presented largely by staff members. These were concerned mainly with discussions of work in progress. Several of the talks were presented by outside speakers. These included the following:

"Stability relations in terms of pH and Eh (oxidation potential)," by R. M. Garrels (U. S. Geological Survey).

"Ionization potentials and the chemical and geochemical properties of the elements," by L. H. Ahrens (Massachusetts Institute of Technology).

"Long-range-short-range order in calcic plagioclases and geologic implications," by F. Laves (University of Chicago).

"Some observations on K-feldspars intermediate between microcline and sanidine," by J. R. Goldsmith (University of Chicago).

"A eutectic system and its molecular-kinetic interpretation," by L. O. Nicolaysen (Princeton University).

"Symposium on the clay minerals," conducted by W. F. Bradley (Illinois Geological Survey) and C. S. Ross (U. S. Geological Survey).



"Relation between freezing-point curves and heats of melting," by L. H. Adams (Carnegie Institution of Washington).

"Strontium isotopes in carbonates," by P. Gast (Department of Terrestrial Magnetism).

"Crystallographic properties of the spinels," by L. R. Maxwell (Naval Ordnance Laboratory).

"The interpretation of a seismogram," by H. E. Tatel (Department of Terrestrial Magnetism).

*Lectures.* During the report year staff members were invited to present lectures as follows:

P. H. Abelson lectured at the Department of Microbiology, Yale University; Journal Club of the Department of Geology, Johns Hopkins University; and Gordon Research Conference, American Association for the Advancement of Science.

F. Chayes lectured at the Symposium of the American Statistical Association.

G. L. Davis lectured at the Lamont Geological Observatory, Columbia University.

G. W. Morey lectured at Oak Ridge National Laboratory; Ontario Research

Foundation; Alfred University; Argonne National Laboratories; and Harvard University.

J. F. Schairer gave a series of lectures at the University of California (Berkeley); University of California at Los Angeles; Stanford University; and California Institute of Technology.

H. S. Yoder, Jr. lectured at Harvard University and Department of Geology, Indiana University.

*Washington Crystal Colloquium.* The Laboratory acted as host for the series of meetings of the Washington Crystal Colloquium held during the report year.

*Co-operative work.* A single-crystal and powder X-ray study of alkali feldspars from the Bear Paw Mountains, Montana, for Dr. W. T. Pecora, of the U. S. Geological Survey, was made by MacKenzie and Smith. Mr. Gordon Burley, of the Constitution and Microstructure Section of the National Bureau of Standards (formerly assistant to Kracek), used the Laboratory X-ray equipment for single-crystal investigations.

The "Summary of Published Work" below briefly describes the papers published in scientific journals during the report year. In addition, the following papers are now prepared for publication: F. Chayes, "Potash feldspar as a by-product of the biotite-chlorite transformation"; F. Chayes, "The lognormal distribution of the elements: A discussion"; F. Chayes, "Effect of change of origin on mean and variance of two-dimensional fabrics: A discussion"; W. S. MacKenzie, "The orthoclase-microcline inversion"; W. S. MacKenzie and J. V. Smith, "The alkali feldspars. I. Orthoclase-microperthites"; G. W. Morey, "The binary systems  $\text{NaPO}_3$ — $\text{KPO}_3$  and

$\text{K}_4\text{P}_2\text{O}_7$ — $\text{KPO}_3$ "; G. W. Morey, "Silica and inorganic silicates"; J. F. Schairer, "The system  $\text{K}_2\text{O}$ — $\text{MgO}$ — $\text{Al}_2\text{O}_3$ — $\text{SiO}_2$ . I. Results of quenching experiments on four joins in the tetrahedron cordierite—ferrosterite—leucite—silica, and on the join cordierite—mullite—potash feldspar"; J. V. Smith, "A review of the Al—O and Si—O distances"; J. V. Smith and W. S. MacKenzie, "The alkali feldspars. II. A simple X-ray technique for the study of alkali feldspars"; J. V. Smith and Th. G. Sahama, "Determination of the composition of natural nephelines by an X-ray method"; H. S. Yoder and H. P. Eugster, "Phlogopite synthesis and stability range."

## SUMMARY OF PUBLISHED WORK

(1208) Reexamination of the crystal structure of melilite. J. V. Smith. *Amer. Mineralogist*, vol. 38, pp. 643-661 (1953).

An accurate single-crystal investigation of an intermediate melilite has confirmed the structure found by Warren in 1930. Variations in O-O distances are explained by sharing of polyhedron edges. Variations in Si-O distances are not explained by cation-cation repulsion. There is a considerable deviation from Pauling's rule of electrostatic valence bonds. This deviation may be related to the variation in Si-O distances, since the longer the distance, the larger is the sum of electrostatic valence bonds. The Ca polyhedron is a fairly regular square antiprism. The Ca-O distances are in two groups at 2.4 Å and 2.7 Å, a fact which suggests that the bonds have different characters. A discussion of the possible arrangements of Mg, Al, and Si atoms in the melilite series is given. Consideration of some new analyses on natural melilites confirms the accepted melilite formula.

(1209) Improved hydrothermal quenching apparatus. J. Van den Heurk. *Bull. Geol. Soc. Amer.*, vol. 64, pp. 993-996 (1953).

There is increasing interest in the study of the role of volatiles in petrological problems. The high cost of the necessary apparatus has restricted such studies. A simplified design for the hydrothermal quenching apparatus initially developed by Tuttle reduces this cost manyfold. The apparatus can be built by a novice using only a drill press, saw, and light lathe. All the materials are available commercially. The pressure is retained at the principal union in the highest temperature zone by a cone-in-cone seal held together by a weighted cantilever. The temperature is attained by a multiple-unit, split furnace. A rapid quench is obtained by an air blast directed at all sides of the pressure vessel. The working range remains 30,000 psi and 1000° C. Water, carbon dioxide, and oil have been used successfully as pressure mediums. The small size, low cost, simple construction, and ease of operation of this apparatus should promote the experimental investigation of the role of volatiles in petrological problems.

(1210) Hydrothermal synthesis. G. W. Morey. *Jour. Amer. Ceram. Soc.*, vol. 36, pp. 279-285 (1953).

The underlying theory of hydrothermal synthesis is presented, and the apparatus used is described. By way of illustration, the determination of the solubility of quartz in superheated steam at high pressures and the determination of the compositions of coexisting gas and liquid phases in the system  $\text{H}_2\text{O}-\text{Na}_2\text{O}-\text{SiO}_2$  at 400° C are discussed.

(1211) In defense of the second decimal. F. Chayes. *Amer. Mineralogist*, vol. 38, pp. 784-793 (1953).

Synthetic and practical examples are used to show that excessive rounding must be avoided whenever quantitative results are to be subjected to extended numerical analysis. In work reporting original measurements it is usually preferable to record the complete observation. Where this is not desirable, it should always be possible for an interested reader to reclaim the original observations. This cannot be done if the hundredths place is rounded out of conventional silicate analyses, or the tenths place is rounded out of modal analyses.

(1212) Studies of the earth's crust using waves from explosions. H. E. Tatel, L. H. Adams, and M. A. Tuve. *Proc. Amer. Philos. Soc.*, vol. 97, pp. 658-669 (1953).

Summaries of the principal seismic data for several continental regions in the United States are presented. The crustal discontinuity was located in three eastern regions but not in two west coast localities. No evidence for any systematic intermediate crustal layers was found. Multiple seismograms are presented to show the peculiar semicoherent pattern of the seismic reverberation.

(1213) The system  $\text{H}_2\text{O}-\text{NaPO}_3$ . G. W. Morey. *Jour. Amer. Chem. Soc.*, vol. 75, pp. 5794-5797 (1953).

The solubility relations in the system  $\text{H}_2\text{O}-\text{NaPO}_3$  up to the melting point of  $\text{NaPO}_3$  were determined by the closed-tube method, and the vapor pressures of the saturated solutions at the invariant points and at 400° and



500° C were also measured. The incongruent melting point of sodium dihydrogen orthophosphate,  $\text{NaH}_2\text{PO}_4$ , at which the reaction  $\text{NaH}_2\text{PO}_4 \rightleftharpoons \text{G} + \text{L} + \text{Na}_2\text{H}_2\text{PO}_4$  takes place, is at 169° C. The incongruent melting point of  $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$ , at which the reaction  $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7 \rightleftharpoons \text{G} + \text{L} + \text{NaPO}_3\text{II}$  takes place, is at 343° C. The inversion of  $\text{NaPO}_3\text{II}$  to  $\text{NaPO}_3\text{I}$  is at 443°, and the vapor pressure of the saturated solution at this quadruple point  $\text{G} + \text{L} + \text{NaPO}_3\text{II} + \text{NaPO}_3\text{I}$  is 15 bars.

- (1214) The theory of thin-section analysis. F. Chayes. *Jour. Geol.*, vol. 62, pp. 92-101 (1954).

A thin-section analysis provides an unbiased estimate of the areal composition of the surface measured. A randomly chosen area provides an unbiased estimate of composition by volume. Orientation of the rock or of the thin section with regard to some structural element of the rock may improve or reduce the efficiency of the sampling, but will not in itself bias the result. The tradition that only unoriented or weakly oriented rocks are suitable for modal analysis is unfounded.

- (1215) Annual report of the Director for 1952-1953.
- (1216) The granite problem: Evidence from the quartz and feldspar of a Tertiary granite. O. F. Tuttle and M. L. Keith. *Geol. Mag.*, vol. 91, pp. 61-72 (1954).

The inversion temperatures of the quartz from 25 specimens of the Beinn an Dubhaich granite have been measured and found to correspond to the inversion temperatures of quartz from rhyolites previously studied. Optical and X-ray studies of the alkali and plagioclase feldspars indicate high-temperature modifications. The Beinn an Dubhaich granite has the textural and compositional features of a granite together with mineralogical features normally associated with rhyolitic rocks. The results serve to emphasize the fact that methods are now available which make it possible in some cases to determine whether a particular granite was formed by metamorphism of sediments or by crystallization of a magma. It is concluded that the older granites and those which crystallized at greater depths probably have undergone considerable rearrangement of their mineralogical and tex-

tural features subsequent to primary crystallization.

- (1217) A test of the revised determinative chart for plagioclase. F. Chayes. *Amer. Jour. Sci.*, vol. 252, pp. 172-180 (1954).

New data on the composition and refractive indices of selected plagioclases, recently published by R. C. Emmons and co-workers, are used to test a determinative table presented by the author in 1952. The table seems to be adequate for practical petrographic work, but the possibility that it or the new data contain small systematic biases cannot be excluded. What evidence there is suggests that the bias, if any exists, is located in the new data.

- (1218) Further complexities in the lamellar structure of alkali feldspars. J. V. Smith and W. S. MacKenzie. *Acta crystallogr.*, vol. 7, p. 380 (1954).

A further study of the lamellar structure of a series of alkali feldspars has revealed new complexities. It has been shown that high-albite and low-albite may each have albite and pericline twinning. One specimen consists of a monoclinic potash phase, a triclinic potash phase, and an albite-twinned soda phase. A simple but accurate method has been developed for the determination of  $\alpha^*$  and  $\gamma^*$  for albite- and pericline-twinned phases from *b*-axis oscillation photographs. Full details will be given in a later paper.

- (1219) Table 120.—Densities and melting and boiling points of inorganic compounds. Table 124.—Reversible transitions in crystals. F. C. Kracek. *Smithsonian Physical Tables*, 9th rev. ed., pp. 120-129 (1954).

This is a compilation of the densities and melting and boiling points for a number of selected inorganic compounds, and the values of transition temperatures at specified pressures, together with values of the heats of transition and transition volume change for most of the compounds that are known to undergo reversible phase transformation in the solid state.

- (1220) The preparation of specimens for the focusing-type X-ray spectrometer. L. H. Adams and F. A. Rowe. *Amer. Mineralogist*, vol. 39, pp. 215-221 (1954).

Following a discussion of the requirements for mounts that will yield high precision for

X-ray diffraction spectra obtained with the focusing-type spectrometer, a method is described for preparing specimens pressed into metal holders, and details are given concerning a suitable press with a rectangular mold. A finely powdered material is mixed with a small amount of Lucite solution, and after drying and crushing it is heated and pressed so as to obtain a flat surface in proper registry with the metal mount. Samples prepared in this way will permit full advantage to be taken of the remarkable precision of which modern spectrometers are capable.

(1221) Experiment as an aid to the understanding of the natural world. N. L. Bowen. *Proc. Acad. Nat. Sci. Philadelphia*, vol. 106, pp. 1-12 (1954).

Experimentally determined phase equilibrium diagrams of anhydrous silicate systems demonstrate that, whatever rock-forming oxides may be present, the final residual liquid from fractional crystallization will be an alkali-alumina-silica-rich liquid nearly free of all other rock-forming oxides except iron oxides, which may still be present in significant amounts. The contention that there will be absolute enrichment in iron oxides in final

residual liquids is not supported by the experimental results.

An investigation of alkali-alumina-silica mixtures, both dry and in the presence of water, shows where the residual liquid from a mixture of all rock-forming oxides lies: it is a liquid consisting of approximately equal parts by weight of soda feldspar, potash feldspar, and silica. If granites are really, as has been supposed, the result of crystallization from a liquid which is the residuum from fractional crystallization of complex liquids containing notable amounts of all the rock-forming oxides, the composition of granites should tend to cluster about this experimentally determined composition. When the compositions of 571 analyzed granites and syenites are plotted in terms of the components soda feldspar, potash feldspar, and silica (more than 80 per cent of their composition is made up of these components), nearly all of them lie in an area close to the experimentally determined position. Such a clustering of the composition of granites is strong confirmation of the hypothesis of their origin by crystallization of the liquid which is a residuum of fractional crystallization.

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## DEPARTMENT OF PLANT BIOLOGY

*Stanford, California*

C. STACY FRENCH, *Director*

One of the interesting discoveries of the range-grass program during the past year was that the small percentage of sexual plants which are always found in the apomictic population may be greatly increased by changing the growth conditions. Thus, strains which maintain nearly pure stands of the apomictic species under favorable growth conditions will sometimes, under unfavorable conditions, show a predominance of the sexual forms, which vary greatly from one to another. A mechanism exists whereby normally suppressed variants may be called forth under changed growth conditions and make possible the survival of a changed kind of bluegrass. A corollary of this observation is that seed production for the apomictic species should be undertaken only under conditions of vigorous growth; otherwise, the seed produced may be variable. Such difficulties are occasionally encountered in seed production even with widely used and well tested varieties of commercial grasses.

The co-operative undertaking with the U. S. Soil Conservation Service continues. The selected strains of grass which last year were considered to be of some promise for practical applications have held up well during the past year, and seed increase of many of them has been undertaken. Furthermore, during the past year the methods of breeding apomictic plants which have been developed during this program have become more thoroughly established as of considerable practical importance. About twenty of the promising strains in 120-foot rows at Pullman have come into seed production this year. Three strains have been planted for seed increase in plots of approximately one-half acre each at Pleasanton, and two strains in approximately the same acreage have been set out by the Ferry-Morse Seed Company for seed increase. One of these may be useful

for turf purposes and the other for range reseeding. The promising strains are listed in a later section of this report, with the different environments and uses for which they appear to be suitable. The major part of the seed to be harvested this fall is committed for use in a climatic screening test by the U. S. Agricultural Research Service. This test, under the supervision of Dr. A. A. Hanson in co-operation with various state agencies, should give a good indication of the suitability of the various strains for use in the major climatic regions of the country.

The work on climatic races within species has been extended during the year to a comprehensive study of the family Lemnaceae by Dr. Landolt, from Zurich. This family, the duckweeds, includes the simplest known forms of flowering plants. These small, rapidly growing plants offer great practical advantages in the experimental elucidation of the nature of climatic races, since their growth under many different conditions may be studied within a single year. The studies have already shown different growth rates under constant conditions for four strains originating from different geographical localities.

During Dr. Clausen's visit to South America several Brazilian biologists expressed interest in establishing an experimental program on evolutionary differentiation in plants in a tropical region. The project was discussed informally among biologists from Rio de Janeiro and the state of São Paulo on an excursion to Mount Itatiaia on the border between the two states and at the National Museum in Rio de Janeiro. Plans were laid for the start of an investigation of selected groups of plants along a transect from Mount Itatiaia eastward toward the Atlantic coast, to be patterned after the investigations of our Department in central California. In



addition to its significance in relation to basic science, it was believed that such a program eventually might be of value in connection with the utilization and conservation of Brazil's natural resources.

In last year's annual report the recently discovered changes in the absorption spectrum of bacteriochlorophyll during its normal function in photosynthesis were mentioned. These experiments have been continued by Dr. Duysens during the past year. In addition to the changes just mentioned, the participation of a cytochrome-like enzyme in the photochemical part of the photosynthetic system has been discovered.

Investigators of photosynthesis have long hoped for direct methods of observing changes in the component parts of the photosynthetic system of a plant while the system is actually in operation. It appears that these optical methods for observing such changes without disturbing the functioning of the process may in time elucidate the mechanism by which energy is transformed from sunlight into stored chemical energy in the form of carbon compounds. The path of carbon in photosynthesis has received great attention in several different laboratories during the past decade, and the course of its transformation from free carbon dioxide to carbohydrates is reasonably well known. The mechanism by which the energy is made available for such transformations of carbon dioxide has, however, remained less accessible to experimental attack. It is hoped that these recent discoveries of Dr. Duysens may eventually be extended to the point where the sequence of the reactions and the nature of many of the substances involved will be clearly delineated. Absorption changes have been observed during the past year by Professor Lundegårdh at Ultuna, Sweden, in green photosynthetic leaves and algae. It appears that the next detectable step after the activation of chlorophyll by light is the oxidation of a cytochrome by chlorophyll. So far, the effects have been studied only in regard to

the spectral changes before and after illumination, but work on the time course of such changes will in all probability be done. Changes in the spectrum of *Chlorella* caused by illumination have been found by Dr. Duysens to show differences in absorption due to substances which have not yet been identified. This field has indeed been barely entered, but appears to hold considerable promise for future development in the study of the photochemical part of the photosynthetic system, the presence of which makes the essential difference between green plants and other living organisms. The small size of these changes makes their measurement rather difficult, and it looks as though the detailed study of these phenomena would occupy many investigators for many years. A new type of recording spectrophotometer is being developed in the Department in the hope of facilitating such experiments.

Investigations of the functioning, and of the mechanics of formation, of chlorophyll in photosynthetic plants require the use of suitable analytical methods for determining the amounts of the various chlorophylls present in different kinds of living tissue. Dr. Smith and Mr. Benitez have just completed a comprehensive survey of such methods based not only on the present practices, but also on newly developed procedures. As a part of this work they have redetermined with considerably higher precision the basic constants on which the spectrophotometric methods for the determination of the concentration of chlorophylls *a* and *b* depend. Absolute absorption spectra for chlorophylls *c* and *d*, bacteriochlorophyll, and their magnesium-free derivatives, the pheophytins, have been made during the past year. Such data are of as fundamental importance to the plant physiologist as are molecular weights to the analytical chemist.

The general program of the study of fluorescence spectra of chloroplast pigments has been continued during the past

year more or less in accordance with the discussion in last year's report. During this time the fluorescence spectra for the purified chlorophylls prepared by Dr. Smith and Mr. Benitez have been determined and a survey has been made of the fluorescent properties of various plants and of their structures. The transformation of protochlorophyll to chlorophyll has been studied by Dr. Virgin, using measure-

ments of the fluorescence spectrum of the intact leaf, in the course of an investigation of the effects of flashing light on this transformation. He has also made a study of the effects of internal scattering within the organism on the shape of the fluorescence spectrum of the pigments contained therein. Infiltration by water greatly reduces the errors from this cause in the leaves of higher plants.

## PERSONNEL

### BIOCHEMICAL INVESTIGATIONS

*Staff:* C. Stacy French, *Director*, Harold W. Milner, James H. C. Smith, Herman A. Spoehr,<sup>1</sup> *Chairman Emeritus*  
*Visiting Investigator:* Hemming I. Virgin  
*Research Fellows:* L. N. M. Duysens, Robert W. Krauss  
*Research Assistants:* Allen Benitez, Jeanette S. Brown, Arthur T. Giese  
*Technical Assistants:* Allen B. Church, Walter W. Holt, Willard C. Sperry, James J. Stekert  
*Stanford University graduate student associated with the Department:* Richard W. Eppley

### EXPERIMENTAL TAXONOMY

*Staff:* Jens C. Clausen, Paul Grun,<sup>2</sup> William M. Hiesey  
*Visiting Investigator:* Elias Landolt  
*Research Assistants:* Charles T. Mason, Jr., Malcolm A. Nobs, Thomas R. Pray  
*Technical Assistants:* Robert W. Ayres, Arthur L. Hawk, John F. Hansen, Edward L. Triplett  
*Clerical Assistants:* John M. Bracken, Alberta B. Caswell  
*Gardener:* Wesley B. Justice

### RESEARCH ASSOCIATE

Ralph W. Chaney, Professor of Paleontology, University of California, Berkeley

### DEPARTMENT SECRETARY

Wilbur A. Pestell

### MECHANICAL ENGINEER

Louis R. Kruger

### CUSTODIANS

Donald B. Muir, Richard P. Ludolph, Jr., Webster L. Smalley

Dr. Herman A. Spoehr, retired Chairman, died on June 21, 1954. Although retired in 1950, he was still active in experimental investigations and gave a great deal of time to correspondence and bibliographic work relating to algal culture.

Dr. Paul Grun resigned March 31, 1954, to become Assistant Professor of Genetics at The Pennsylvania State University.

Professor Henrik Lundegardh of Uppsala, Sweden, conferred with the staff for several days on the changes in absorption spectra of illuminated photosynthetic cells, and visited the gardens at Mather.

Dr. C. J. P. Spruit of Wageningen, Netherlands, was here a few weeks investigating the production of light at electrodes in chlorophyll solutions.

Dr. Robert Airth of the Hopkins Marine Station used the fluorescence equipment on various occasions to study solutions of phycobilin pigments.

Drs. James H. C. Smith and Herman A. Spoehr assisted Professors Howard E. McMinn and Richard Wistar of Mills College in the preparation of an educational exhibit on chlorophyll for the Oakland Flower Show.

Dr. Th. Dobzhansky of Columbia University is utilizing the facilities at the Mather and Timberline transplant stations in a co-operative study of climatic races of *Drosophila*, with special emphasis on food specialization and its adaptive importance.

<sup>1</sup> Died June 21, 1954.

<sup>2</sup> Resigned March 31, 1954.



Co-operators in this program include Dr. Hampton Carson, Washington University, St. Louis; Drs. H. Pfaff and E. Mrak, University of California, Davis; Dr. E. Knapp, University of São Paulo, Brazil; and Mr. D. Cooper, Columbia University,

New York. Studies of ecotypic differentiation within *Drosophila pseudoobscura* and *D. persimilis* along the altitudinal transect of the Sierra Nevada, begun in 1945 by Dr. Dobzhansky, are being continued.

### EXPERIMENTAL TAXONOMY

The projects in experimental taxonomy are all aimed at improving our understanding of various phases of the dynamics of evolution in plants. Of necessity, they extend over long periods of time, and for practical reasons it is desirable to have running concurrently several lines of investigation that are in different stages of maturation.

A new project, a study of the biology of the Lemnaceae or duckweed family, was begun during the year by Dr. Elias Landolt of Zurich, Switzerland, who is with the Department as a visiting investigator. The members of the Lemnaceae are world-wide in distribution and include the smallest of the flowering plants. They are well suited for studies on comparative physiology of ecologically distinct races and species, and can be grown rapidly as clones in pure culture in test tubes or flasks.

Other projects described in previous Year Books have been continued, and in some of these, significant developments have taken place. Work on major manuscripts dealing with completed long-range investigations has advanced. The one currently at the focus of attention deals with the nature of the genetic structure that distinguishes climatic races of a single diploid species, *Potentilla glandulosa*. Experimental data on the Madiinae, dealing primarily with the evolution of species barriers in a group consisting of about 80 species in 6 major genera, remain to be analyzed and presented for publication (see Year Book No. 40, 1940-1941, pp. 160-169).

The bluegrass (*Poa*) project, although approaching maturity, still requires a

major proportion of the efforts of the staff. Through the *Poa* investigations, our knowledge of the hereditary structure and evolutionary patterns within one of the most complex polyploid and largely apomictic genera of higher plants has been greatly extended. This information will be summarized in a monograph which may have wide practical significance in establishing principles for plant breeding in apomictic plant groups. At the nurseries of the U. S. Soil Conservation Service at Pleasanton, California, and Pullman, Washington, the established stands of numerous hybrid bluegrasses that have been developed for agronomic purposes during the past ten years are still under study.

A study begun in 1947 of the hereditary basis of ecological races and species in *Achillea*, of the sunflower family, has advanced during the year with the development of new hybrids and  $F_2$  populations needed for analytical study. The studies on *Achillea* throw much light on the nature of inheritance in contrasting climatic races in polyploid species. Some aspects of the *Achillea* studies are being carried on in co-operation with Dr. Friedrich Ehrendorfer of Vienna, Austria, who worked as a visiting investigator at our Department during the summer of 1952. The central European members of this complex include species on all chromosomal levels ranging from diploid to octoploid, and the relations between these may be the key to an understanding of the structure of the North American members of the complex, which include only tetra- and hexaploids.

For a number of years the group in experimental taxonomy has been searching

for an ideal experimental organism for critical studies on the relations between morphological and physiological gene-controlled characteristics that have significance in natural selection for distinct environments. This matter was discussed eight years ago at a group conference held at Mather (see Year Book No. 46, 1946-1947, pp. 103-104). The studies to date on the *Mimulus cardinalis-lewisii* complex, mentioned in Year Book No. 50 (1950-1951, pp. 118-119), now clearly indicate that this group meets very closely the exacting specifications for an ideal organism for experiments of this scope.

Progress has been made in the development of apparatus for the precise measurement of rates of photosynthesis and of respiration of plants under controlled conditions, but the final arrangement and assembly of the parts remains to be completed. The primary objective is to build a serviceable and accurate analytical system that can be used in comparing the basic metabolism of contrasting ecologic races or species of different plant groups under a wide range of controlled conditions. These physiological studies will be closely integrated with the genetic, cytological, and field investigations of the various groups of plants mentioned above.

Dr. Paul Grun returned in October 1953 from Stockholm, Sweden, where he spent a year with Professor T. O. Caspersson of the Karolinska Institutet, studying the nucleic acid and protein content of nuclei of root-tip cells of *Tradescantia*. The results indicated that whereas the nucleic acid content of cell nuclei increases during the period between cell divisions (interphase), the protein content probably increases during the actual stages of division. A manuscript for a paper on these studies has been prepared. On his return, Grun completed three manuscripts of papers on his researches on *Poa*, including one on chromosome numbers in interspecific hybrids, another on frequencies of univalents in species and interspecific hybrids, and a third on variation within *Poa*

*nervosa*, an obligate apomictic species. These papers have been submitted for publication.

Dr. Clausen was abroad in the late summer and fall of 1953. He participated in the International Symposium on Population Genetics in Pavia, Italy, and the Ninth International Genetics Congress in Bellagio, Italy. During October and November he visited universities and scientific institutions in Brazil, located in Porto Alegre, Rio Grande do Sul; Curitiba, Paraná; São Paulo, Campinas, and Piracicaba in São Paulo; Rio de Janeiro, D. F.; Belo Horizonte, Minas Gerais; and Belém do Pará, Pará. The visit to Brazil was prompted by an invitation from the Cultural Division of the Brazilian Ministry of Foreign Relations as part of a Brazilian program for international exchange of scholars. At the various institutions he lectured on principles of variation and evolution of plants and conferred with small groups of biologists, discussing their projects. Throughout the length of the country, in company with Brazilian biologists, he took part in a number of excursions to contrasting types of plant communities for the purpose of studying these communities in relation to our understanding of evolution.

Several Brazilian biologists from Rio de Janeiro and the state of São Paulo were interested in initiating a co-operative experimental program on the study of evolutionary differentiation in plants in a tropical region. Preliminary plans were laid for starting an investigation on selected plant groups along a Brazilian transect from Mount Itatiaia on the border between the states of Rio de Janeiro and São Paulo eastward toward the Atlantic. Topographically, this transect has some of the characteristics of our transect in central California.

#### THE POA PROGRAM

JENS CLAUSEN, WILLIAM M. HIESEY, AND  
MALCOLM A. NOBS

In the partially apomictic Poas two modes of reproduction are in balance, the



asexual, or apomictic, type, and the sexual. In most species of *Poa* the latter is normally suppressed (see Year Book No. 46, 1946-1947, pp. 101-103). Evidence accumulated during the year now makes it clear that environment may shift the direction of this balance. Normally, strains of *Poa* grown in their native environment produce maternal, apomictic offspring that are vigorous, and some sexually reproduced progeny that appear as weak aberrant types. If the plants are brought into a very different environment, the relative vigor of the apomictic and sexual offspring may be altered markedly, or even reversed.

Earlier studies of the hybrid apomictic strains of *Poa* were made on space-planted seedlings in experimental gardens. This was essential in order to study the relative proportions of asexual and sexually reproduced progeny in a given strain. During 1953 approximately 24 of the established apomictic hybrids were sown thickly in drilled rows in field plots at the Pleasanton, California, and Pullman, Washington, nurseries of the Soil Conservation Service, and also at Davis, California, in sowings made by the Department of Agronomy at the University of California. The young seedlings thus closely crowded together were subject to intense competition. The significant discovery was made that the extent of elimination of sexual aberrants in such densely sown field rows may differ strikingly in the same apomictic strain when it is grown in different environments.

In the California plantings of one of the most promising  $F_1$  lines, for example, the apomictic progeny are more vigorous than the sexual aberrants, which are scarcely observable; but at Pullman the aberrants are the stronger and become highly conspicuous in the drilled row plantings. This situation was observed in line 4535-6, an  $F_1$  hybrid between *Poa ampla* from Albion, Washington, and a form of *P. pratensis* originally from the central Sierra Nevada. Moreover, a sister line derived from the same parents, line 4535-26, reacts in the

opposite direction, the apomictic type growing poorly in California and being overshadowed by the sexual aberrants, whereas at Pullman it shows so much vigor that the variants are largely eliminated. These two sister lines originally from the same parentage have very different ranges of tolerance in these two environments, one being clearly suited to California conditions, and the other to the Pacific Northwest. In the production of seed, the purity of each strain will obviously depend on the degree to which the sexual aberrants are eliminated.

A similar observation was made on an  $F_1$  line of a hybrid between *Poa scabrella* and *P. ampla* (line 4569-1). The *scabrella* parent is native to the Coast Ranges of southern California, whereas the *ampla* parent was originally collected from Kahlottus, near the Snake River, in southeastern Washington. The  $F_1$  hybrid is approximately 65 per cent apomictic and well suited for growth in the Coast Ranges of California, where it is winter-active and considerably more productive than either parent. In the California plantings only the apomictic-type seedlings are observable in drilled row plantings, the sexual progeny being practically eliminated by competition. At Pullman this same strain produces leafy rosettes that develop early in spring, but seed production on these is poor as compared with that on sexual aberrants. It is evident that commercially the seed of such a strain would have to be produced in California.

An isolated example of differential vigor of apomicts and sexual-type plants had previously been observed in a wild strain of *Poa pratensis*. A strain native at Timberline station at 10,000 feet altitude in the Sierra Nevada produced apomictic seedlings that in the lowland environment at Stanford grew poorly and became highly diseased. Some of the aberrants, however, surpassed the apomictic type at Stanford, and one plus variant of this type has been able to survive there for 9 years. It is about 50 per cent apomictic, a fact which

indicates that the high-altitude race of *P. pratensis* contains genetic potentialities from which new strains may be selected in different kinds of environment. This alpine strain also produces plus aberrants in the Pullman environment.

This profound influence of environment on relative vigor implies that there is a dynamic equilibrium between the apomictic type surviving in the native habitats and the potential sexual variability in the germ plasm of these highly polyploid races and species of *Poa*. In the event that the type of habitat is drastically changed, the balance relations between the apomictic and the sexual fractions of the progeny may be changed. Such a mechanism of partial apomixis seems to be an ideal one for insuring the survival of the species in the face of changing environments.

It is becoming increasingly clear that in *Poa* the potentialities for producing a great range of new apomictic strains through intersectional hybridization are almost unlimited. An illustration is provided by a family of strains derived from a single cross between *P. ampla* from Kahlotus, southeastern Washington, and a strain of *P. pratensis* originally from the Athabaska region of central Canada, known as the "Delta" strain. This cross yielded 21  $F_1$  hybrids, but only 3 of these were vigorous enough to produce sufficient progeny for testing. These 3  $F_2$  progenies were sexual and highly variable. One of the  $F_1$  plants produced enough seed to plant 240  $F_2$  individuals in each of six localities, namely, Stanford, Pullman, Edinburgh (Scotland), Ötoftgaard (Denmark), Uppsala (Sweden), and Volbu (Norway). At Pullman the progeny of 15 selected  $F_2$  individuals were tested, and among them five promising apomictic lines were found. These five lines were included in dense drill row sowings at Pleasanton and Davis in California and at Pullman, Washington. In these tests the *ampla* parent is weak-stemmed and tends to fall, and is highly susceptible to rust, especially in California. The *pratensis* parent also tends to fall and

is likewise relatively stemmy. All five hybrid lines are improvements over the parental types, but each is distinctive. Two are erect-stemmed, leafy, rust-resistant, and late-flowering types that would ordinarily be classed as *P. pratensis*; one is a moderately rhizomatous type that is highly productive at Pullman but decidedly less so in California; another is a tall, early strain that tends to be weak-stemmed but has excellent autumn recovery, and a fifth line is intermediate between the extremes. Two strains appear to fit both the central California and Palouse Prairie conditions. In addition, two apomictic strains have been derived from  $F_2$  progeny selected at Edinburgh, and seven more from  $F_2$  progeny grown at Stanford. The characteristics of the latter nine strains have not yet been adequately studied.

Besides these 14 stabilized apomictic derivatives from this single cross, progeny from another individual of the same origin continues to segregate widely through the  $F_4$  generation, ranging from highly rhizomatous to bunch-type, highly leafy to extremely stemmy, short to tall, almost horizontal to erect, and blue-glaucous to green-leaved plants in different recombinations. The range of variability within a single  $F_4$  progeny reveals something of the extent of potential variability stored in the germ plasm of the parental strains.

From the accumulated observations made on the hybrid *Poa* lines in various environments, it seems likely that as many as 10 or 15 of the approximately 45 stabilized hybrid lines may eventually find places in the agricultural economy of our western states. All these strains have been derived from crosses between taxonomically distinct sections of the genus.

It now seems possible to classify provisionally the older experimental strains according to agronomic use groups, as listed in table 1. The classification also takes into account the climatic region for which the strain is adapted, and the season during which it can be used. The same strain may serve different uses in different sections of



TABLE 1

USE GROUPS OF HYBRID POA STRAINS FOR THE COAST RANGES, VALLEYS, AND FOOTHILLS OF CENTRAL CALIFORNIA AND FOR THE PALOUSE PRAIRIE REGION

| LINE NO.  | COMBINATION, ORIGIN, TYPE  | REGION                                      |                        |
|---|--|---|------------------------|
|   |  | Coastal valleys and foothills of California | Palouse Prairie region |
| WINTER OR SPRING GRASSES, coastal valleys and foothills of California only                          |  |   |                        |
| Range types:  |  |   |                        |
| 4569-1  | <i>scabrella-ampla</i> , Posas-Kahlotus, bunch   | x   | o                      |
| 4711-3  | <i>scabrella-pratensis</i> , Posas-Mather, rhizomatous   | x   | o                      |
| 4724-4  | <i>scabrella-pratensis</i> , Posas-Leevining, semi-bunch   | x   | o                      |
| 13548-10  | <i>scabrella-pratensis</i> , Posas-Leevining, rhizomatous  | x   | o                      |
| WINTER AND SUMMER GRASSES in California, or SPRING AND EARLY SUMMER TYPES in Palouse Prairie region |  |   |                        |
| Range types:  |  |   |                        |
| 4681-3  | <i>ampla-pratensis</i> , Condon spontaneous, short rhizomes  | x   | x                      |
| 4729-11   | <i>ampla-pratensis</i> , Albion-Athabaska, short rhizomes  | x   | o                      |
| Pasture types:  |  |   |                        |
| 4683-1  | <i>ampla-alpigena</i> , Albion-Lapland, rhizomatous  | x   | o                      |
| 4684-5  | <i>ampla-alpigena</i> , Albion-Lapland, rhizomatous  | o   | x                      |
| 13838-305 or -513   | <i>scabrella-pratensis-alpigena</i> , Posas-Mather-Lapland, rhizomatous; new, tested only in Pacific Northwest | ?   | x                      |
| 13989-308   | <i>ampla-compressa-alpigena</i> , Albion-Chorsum-Lapland, rhizomatous; new, tested only in Pacific Northwest   | o   | x                      |
| SPRING AND SUMMER GRASSES in California, or SUMMER GRASSES in Palouse Prairie region                |  |   |                        |
| Range types:  |  |   |                        |
| 4535-6  | <i>ampla-pratensis</i> , Albion-Mather, short rhizomes, late   | x   | o                      |
| 4535-26   | <i>ampla-pratensis</i> , Albion-Mather, short rhizomes, late   | o   | x                      |
| 13775-202   | <i>ampla-pratensis</i> , Albion-Mather, rhizomatous, tall  | o   | x                      |
| 13783-208   | <i>ampla-pratensis</i> , Kahlotus-Athabaska, short rhizomes  | o   | x                      |
| 13783-301   | <i>ampla-pratensis</i> , Kahlotus-Athabaska, rhizomatous   | x   | x                      |
| 13783-507   | <i>ampla-pratensis</i> , Kahlotus-Athabaska, rhizomatous, tall, early  | x   | x                      |
| Pasture types:  |  |   |                        |
| 13775-202   | <i>ampla-pratensis</i> , Albion-Mather, rhizomatous, tall  | o   | x                      |
| 13783-301   | <i>ampla-pratensis</i> , Kahlotus-Athabaska, rhizomatous, low, fairly late                                     | x   | x                      |
| 13833-211 or -311   | <i>ampla-pratensis</i> , Albion-Mather, rhizomatous, late, very leafy  | o   | x                      |
| 13832-409   | <i>ampla-pratensis</i> , Albion-Mather, rhizomatous, late, very leafy  | x   | o                      |
| VERY LATE RANGE TYPE  |  |   |                        |
| 13950-412   | <i>ampla-compressa</i> , Albion-Chorsum, sexual, short rhizomes  | o   | x                      |
| TURF GRASSES  |  |   |                        |
| 4694-8  | <i>scabrella-pratensis</i> , Posas-Athabaska, winter-active  | x   | o                      |
| 5971-208  | <i>ampla-pratensis</i> , Albion-Mather, fine leaves, new, tested only in Pacific Northwest                     | ?   | x                      |

Preliminary tests: x, successful; o, not successful.

the country, according to its genetic characteristics. The classification presented is a broad one, and because of the extreme diversity of climates in the Pacific coast states, the categorical designations should be regarded as only approximate.

Much work remains to be done in the final testing of the *Poa* strains before agricultural uses can be fully determined. For such tests we are largely dependent on co-operating agencies, especially the U. S. Soil Conservation Service. At the Pleasanton Nursery, half-acre plantings of each of three strains thought to be desirable for central California were sown for seed increase in January 1954. Seed increase plots at Pullman, with row plantings ranging in length from some 60 to 600 linear feet, have come into production during the current summer; all the hybrid strains of promise are represented in these plantings except those discovered during the past two or three years.

Dr. R. Merton Love of the Department of Agronomy of the University of California at Davis is testing 24 hybrid strains and 6 parental lines in replicated row plantings sown in the field at the University farm at Davis. Many of the strains show promise in this environment, which is representative of the Central Valley of California.

The Ferry-Morse Seed Company of Mountain View, California, has been growing two rhizome types of *Poa* in turf test plots for comparison with standard commercial strains. One of these appears to be promising. This strain, line 4694-8, is an F<sub>2</sub> line from a cross between *P. scabrella* from the Coast Ranges of southern California and the "Delta" strain of *P. pratensis* from Canada. This grass is more of a turf type than its *pratensis*-like parent and has inherited the winter-active growth of the *scabrella* parent, an extreme bunch type. This strain and line 4569-1, an F<sub>1</sub> apomictic hybrid between *P. scabrella* and *P. ampla*, are being grown for seed increase by the Ferry-Morse Seed Company in approximately half-acre plots.

A series of preliminary tests in the major climatic regions of the United States is being planned by the U. S. Agricultural Research Service as soon as sufficient seeds are available from the seed increase plantings at Pullman and at Pleasanton. These tests, arranged by Dr. A. A. Hanson of this Service in co-operation with state agencies in the various regions, will probably be started during the fall of 1954 and the spring of 1955. The object of the preliminary tests is to determine whether any of the strains may be successful in regions outside the western states. These plantings should also yield information of considerable value in interpreting the evolutionary patterns in *Poa* and the mechanism by which new races and species have been evolved. Members of the Institution's staff plan to visit and study the plantings in order to secure comparable data and to establish a closer liaison with each of the co-operating investigators in the various regions.

A fellowship for work on grass breeding jointly supported by Purdue University, Lafayette, Indiana, and the Carnegie Institution of Washington is scheduled to be started in the fall of 1954. During the summer of 1953, 44 of the hybrid strains together with parental lines were planted by the Department of Agronomy at Lafayette for general study and comparison. It is conceivable that some of these may be of value in Indiana, even though they were bred for the West. It is expected that the first joint project in the development of new strains for Purdue will be an attempt to breed apomictic strains of the combination *Poa arida* × *P. ampla* and thus combine a heat-tolerant rhizome species of the Great Plains with *P. ampla* from the Pacific Northwest. Considerable background data from earlier attempts to synthesize stabilized apomictic lines of this combination suggest that valuable new hybrids suited for the Great Plains region may thus be produced. Vigorous F<sub>1</sub> hybrids grown by us in various environments show much promise.



Other new developments include shipments of seeds of 41 strains of hybrids and parental species to the Department of Agronomy and Genetics at the University of Minnesota at Minneapolis, where preliminary tests will be conducted by Dr. H. L. Thomas, and of 19 strains to Dr. J. M. Hercus of the Department of Agriculture of New Zealand at Dunedin. In the selection of strains for Minnesota, special attention was paid to strains able to tolerate a severe winter, whereas in the shipment to New Zealand emphasis has been laid on strains suited to milder winters.

#### CHROMOSOME NUMBERS OF HYBRID POA LINES

EDWARD L. TRIPLETT AND JENS CLAUSEN

One of the current projects is to count the number of chromosomes in all the apomictic hybrid lines. Although this task has not yet been completed, certain important facts are already evident. One is that successful hybrid lines more often than not have chromosome numbers that are multiples in polyploid series of the basic number, 7. This conclusion is based on a study of 26 apomictic lines, which are included in table 2. Of these, 20 are either multiples of 7, or numbers adjacent to such multiples. This finding seems to be especially significant in view of the fact that in all these hybrids one of the parents was a form of *Poa pratensis*, a species that has highly variable but nonmultiple chromosome numbers. Some vigorous and successful *Poa* lines listed in table 2 have chromosome numbers intermediate between the multiples of 7, as, for example, two of the *P. scabrella* × *pratensis* F<sub>2</sub> lines having 2*n* chromosome numbers of 66 and 67, respectively, midway between the multiples 63 and 70.

Another significant observation is that each sexual generation in hybrid lines may vary abruptly in chromosome number from the preceding one. Such variations are usually accompanied by major changes in other characteristics. For example, the

F<sub>1</sub> hybrid line 4273-9, *Poa ampla* × *P. pratensis alpigena*, has 63 somatic chromosomes, or 9 times a basic set of 7. This

TABLE 2  
SOMATIC CHROMOSOME NUMBERS IN APOMICTIC  
HYBRID LINES OF POA

| Line no.  | F <sub>1</sub> or<br>F <sub>2</sub> line | 2 <i>n</i> of<br>line | Parent F <sub>1</sub> of the<br>F <sub>2</sub> lines, and 2 <i>n</i> |
|---|--|-----------------------|--|
| <i>Poa ampla</i> , Albion, 2 <i>n</i> =63, × <i>pratensis alpigena</i> , Lapland, 2 <i>n</i> =74: |  |                       |  |
| 4273-9....  | F <sub>1</sub>                           | 63                    | .....  |
| 4684-5....  | F <sub>2</sub>                           | 93                    | 4273-9, 2 <i>n</i> =63   |
| 4683-1....  | F <sub>2</sub>                           | 69-70                 | } 4273-8, 2 <i>n</i> =73   |
| 4683-4....  | F <sub>2</sub>                           | 63                    |  |
| <i>Poa ampla</i> , Albion, 2 <i>n</i> =63, × <i>pratensis</i> , Mather, 2 <i>n</i> =68:           |  |                       |  |
| 4535-6....  | F <sub>1</sub>                           | 95-97                 | .....  |
| 4535-26...  | F <sub>1</sub>                           | ca. 100               | .....  |
| 13775-212..   | F <sub>2</sub>                           | 56-57                 | 4535-4, 2 <i>n</i> =63   |
| 13823-706..   | F <sub>2</sub>                           | 55-57                 | 4535-5, 2 <i>n</i> =93   |
| <i>Poa ampla</i> , Kahlotus, 2 <i>n</i> =64, × <i>pratensis</i> , Athabaska, 2 <i>n</i> =70:      |  |                       |  |
| 13783-29...   | F <sub>2</sub>                           | 93                    | } 4537-9, 2 <i>n</i> =ca. 83   |
| 13783-33...   | F <sub>2</sub>                           | 68                    |  |
| 13783-301..   | F <sub>2</sub>                           | 80                    |  |
| 13783-507..   | F <sub>2</sub>                           | 70-71                 |  |
| <i>Poa ampla</i> , Condon, 2 <i>n</i> =63, × <i>pratensis</i> , spontaneous hybrid:               |  |                       |  |
| 4681-3....  | F <sub>2</sub>                           | 77-80                 | 4173-12, 2 <i>n</i> =97-99   |
| <i>Poa scabrella</i> , Las Posas, 2 <i>n</i> =82-84, × <i>ampla</i> , Kahlotus, 2 <i>n</i> =64:   |  |                       |  |
| 4569-1....  | F <sub>1</sub>                           | 75-76                 | .....  |
| <i>Poa scabrella</i> , Las Posas, 2 <i>n</i> =84, × <i>pratensis</i> , Athabaska, 2 <i>n</i> =70: |  |                       |  |
| 4694-8....  | F <sub>2</sub>                           | 66                    | 4553-2, 2 <i>n</i> =70   |
| <i>Poa scabrella</i> , Las Posas, 2 <i>n</i> =82-84, × <i>pratensis</i> , Mather, 2 <i>n</i> =68: |  |                       |  |
| 4557-3....  | F <sub>1</sub>                           | 75                    | .....  |
| 4711-3....  | F <sub>2</sub>                           | 66-68                 | 4557-10, 2 <i>n</i> =75  |
| <i>Poa scabrella</i> , Las Posas, 2 <i>n</i> =82, × <i>pratensis</i> , Leevining, 2 <i>n</i> =68: |  |                       |  |
| 4559-2....  | F <sub>1</sub>                           | 79-80                 | .....  |
| 4724-4....  | F <sub>2</sub>                           | 99-102                | 4559-3, 2 <i>n</i> =ca. 80   |

hybrid has low fertility although it is apomictic (cf. Year Book No. 46, 1946-1947, p. 99). One of the sexually produced F<sub>2</sub> aberrants from this hybrid was highly

vigorous, fertile, and apomictic, and was found to have  $2n=93$  chromosomes, a gain of 30. This line is of potential agronomic value.

Improvement of an apomictic line may also follow a reduction in chromosome number. A sister  $F_1$  hybrid of the one just mentioned, 4273-8, has  $2n=73$  chromosomes, or 3 beyond the 10-ploid number. This  $F_1$  line is weak and barely survives at Stanford, but occasional vigorous aberrants are produced. Two of these lost 3 and 10 chromosomes, respectively, and gave rise to vigorous, apomictic, and fertile  $F_2$  lines. One of these, the 70-chromosome line 4683-1 (see table 2), is most vigorous in coastal regions over a range of latitudes from  $34^\circ$  to  $61^\circ$  north, whereas the 63-chromosome strain, line 4683-4, is better able to survive in more continental conditions. It is obvious that changes in chromosome number are linked with differences in vigor, fertility, and tolerance to different environments.

Among 57  $F_1$  hybrids originally obtained from the cross *Poa ampla*, Albion ( $2n=63$ )  $\times$  *P. pratensis*, Mather ( $2n=68$ ), many were found to have  $2n=90-93$  chromosomes, and apparently were the result of the union of all the chromosomes of *ampla* and approximately half the chromosomes of the *pratensis* parent. One of these, the stemmy 4535-5, gave rise to two vigorous aberrants, and from one of these a new  $F_2$  strain, 13823-706, was derived which has  $2n=56$  chromosomes. Besides having 37 fewer chromosomes than the parent plant, it is highly leafy and one of the agronomically best *Poa* hybrids ever obtained. It is, however, fairly sterile although apparently apomictic. Another derivative from the same cross, the  $F_2$  line 13775-212, also having the low number  $2n=56$  chromosomes, is highly fertile but only moderately vigorous and fairly stemmy.

The chromosome numbers of two exotic species of *Poa* were determined. One is *P. caespitosa* Forst., a species native to Australia and New Zealand. A strain re-

ceived from Canberra, Australia, was octoploid, having  $2n=\text{ca. } 56$  chromosomes, whereas another strain from Atwater Valley, Marlborough, New Zealand, collected by L. B. Moore at an altitude of 500 feet, had chromosome numbers ranging between the 13- and 14-ploid levels,  $2n=\text{ca. } 90-100$ . The Canberra strain of this bunch grass is sexual, and it can be hybridized with *P. ampla*, *P. pratensis*, *P. compressa*, and *P. arachnifera*, species that have chromosome numbers in the same general range as the Australian *P. caespitosa* (cf. Year Book No. 51, 1951-1952, pp. 112-113).

*Poa iridifolia* Hausmann, described in 1917, is a recently introduced species from Argentina. It grows vigorously in central California and is a tetraploid species with  $2n=28$  chromosomes. This species is a dioecious bunch grass with highly tomentose lemmas and unusually wide leaves. Probably it is related to *Poa arachnifera* of the southern Great Plains of the United States, a dioecious rhizome *Poa* with highly tomentose lemmas.

#### GENETIC STUDIES ON MIMULUS

MALCOLM A. NOBS

The *Mimulus* study is the outcome of a conference held at Mather in 1947 for the purpose of exploring a feasible approach to an understanding of the genetic-physiologic differentiation in plants. In searching for research material meeting the exacting requirements for critical investigation of both the genetic and physiological characteristics of plants occurring in contrasting ecological zones, discussion centered on the possibilities of the genus *Mimulus*. Steps were taken to obtain exploratory materials, and some crossings were made at Stanford the following year. At that time Robert K. Vickery, then a graduate student at Stanford University, began a genetic study of the genus for his doctorate, and through a co-operative arrangement was able to pursue his graduate research at the laboratories of the Department.



Among the numerous hybrid combinations made by Vickery was one between *Mimulus cardinalis* and *M. lewisii*, the  $F_1$  of which proved to be fully fertile and capable of producing a wide array of vigorous recombinations in the  $F_2$  (see Year Book No. 49, 1949-1950, pp. 107-109, and No. 50, 1950-1951, pp. 118-119). Crossings using more widely contrasting forms of the two parent species were made in the years following, and were reported in Year Book No. 52 (1952-1953, p. 175).

This year the background work for future transplant studies on this complex is being carried out. An analysis of the segregation and recombination of about twenty characters which distinguish the parental forms is under way in a second generation of approximately 600 plants growing in the Stanford garden. Extreme segregation is found in the  $F_2$  population, as well as apparently highly complex genetic ratios, suggesting that even simple-appearing characters are controlled by the action of multiple gene systems. A series of selected  $F_2$  plants is being selfed to obtain  $F_3$  generations for a classification of the genetics of such complex systems. The relations of the position of the anthers and of the stigma to the architecture of the corolla, strictly determining the pollinating mechanisms, are among the most interesting sets of characters that are being analyzed. One parent species, *Mimulus cardinalis*, has vermilion-colored, reflexed corolla lobes and a long, narrowly constricted corolla tube with a horizontal opening approximately 4 mm across. The style and stigma are exserted to about 16 mm beyond the corolla, and the anthers are also exserted but are arranged into a flattened, caplike structure about 3 to 4 mm behind the stigma. The arrangement of the corolla, anthers, and stigma is perfectly adapted to pollination by hummingbirds, which feed on this species both under natural conditions and in the garden. As the hummingbird inserts its bill into the corolla tube, its head is dusted with pollen from the caplike anthers. In the next

flower visited, the pollen is brushed from the hummingbird's head to the stigma as the bill of the bird is being thrust into the corolla. Bees and other insects often visit the flowers of *M. cardinalis*, but the stigma is so far exserted that pollination does not occur.

The other parent species, *Mimulus lewisii*, has pink flowers and a slightly shorter corolla tube with erect or slightly spreading corolla lobes. This species has an ample corolla opening 10 to 13 mm across, a short style, and a stigma situated 3 to 4 mm down within the wide corolla tube. The anthers are compressed along the style about 1 mm below the stigma. This kind of flower structure is well adapted to bees, which generally pollinate this species. When the bee crawls into the wide opening of the corolla for nectar, its back becomes dusted with pollen, which it carries to the next flower. As the bee crawls into the tube of the second flower, its back brushes pollen on the stigma. Although hummingbirds occasionally visit the flowers of *M. lewisii*, apparently they do not effect pollination.

The  $F_1$  hybrid combines the characters of the parental forms in having a rose-colored flower with spreading to slightly reflexed corolla lobes. The corolla tube is broad, having an opening 9 to 10 mm across. The style and stigma are exserted well beyond the opening, with the anthers slightly to the rear. For natural fertilization this arrangement appears to be most unfortunate, because neither hummingbirds, bees, nor other insects can effect pollination. With artificial pollination, however, this  $F_1$  is fully as fertile as either parent. Under garden conditions, both at Stanford and at Mather, practically no seed was set on over 200  $F_1$  plants although these plants were constantly visited by bees and occasionally by hummingbirds.

The data from the  $F_2$  population are still incomplete. It appears, however, that only a small number of the  $F_2$  plants can be pollinated naturally. The few reproductively successful  $F_2$  recombinations are of

three main types: (1) those which most closely resemble *M. cardinalis* in both flower color and architecture and are pollinated by hummingbirds; (2) those which approach *M. lewisii* in flower architecture regardless of color and are pollinated by bees; and (3) those which have a rare combination of the shorter style and stigma of *M. lewisii* and the long anthers of *M. cardinalis*, and are self-pollinating.

Although genetically the two parent species react as subspecific entities in that they are interfertile and produce a strong and vigorous  $F_2$  when artificially pollinated, they have imposed upon them a four-way series of barriers preventing free interchange of genes under natural conditions. First, they are geographically and ecologically isolated, *M. cardinalis* occupying the lower elevations from near sea level to approximately 5000 feet, and *M. lewisii* being found at higher elevations. In Yosemite Valley, the two species are found growing together, but progeny tests from plants open-pollinated in this locality produced no hybrids. Another kind of barrier is imposed by the difference in the structure of the flowers, fitting them for different pollinating agents; apparently, this barrier is sufficient to prevent initial hybridization. When hybrids do occur, the third barrier is imposed in the  $F_1$  structure of the flowers, which are not readily fertilized by any of the normal pollinating agents; this in itself would be sufficient to prevent the establishment of large hybrid swarms. Furthermore, even if such hybrids did become established, only those types in succeeding generations which most closely resembled the parental forms would become pollinated, so that selections diverging toward the parental forms would be favored. The only exceptions are those rare recombinations that are self-pollinating, and, conceivably, these might become the progenitors of a new species.

A series of new  $F_1$  hybrids came into flower this year. This new cross is between the same race of *M. cardinalis* from

Los Trancos Creek that was used in the above cross, and a different race of *M. lewisii*, from Stevens Pass, Washington. These  $F_1$  plants have the same structural pattern as those in the earlier cross. They likewise set abundant seed when artificially self-pollinated, but fail to fruit when left to natural pollination.

#### STUDIES ON THE LEMNACEAE

ELIAS LANDOLT

The primary objective of the present study is to explore the general biosystematic structure of the Lemnaceae, a distinctive family of aquatic plants belonging to the monocotyledons. Because of their small size and the relative ease of growing them as clones in pure culture, the members of this family seem to be especially well suited for comparative physiological studies of ecological races and species. One of the aims of this study is to explore their suitability in this regard.

The members of the Lemnaceae are distributed throughout the world except in the arctic and antarctic regions, and occur in a wide range of climates. The plants of all the genera are highly reduced: in *Spirodela*, to fronds bearing several roots; in *Lemna*, to fronds with a single root; in *Wolffia* and *Wolffiella*, to a single frond without roots. Although seeds may occur occasionally, vegetative propagation through budding greatly predominates.

The taxonomy of the family has been studied rather extensively from a descriptive point of view, and there are many papers in the literature dealing with specific physiological investigations. Very little is known, however, concerning the actual delimitation of species, chromosome numbers, or the comparative characteristics of the various genera and species when grown under comparable conditions of culture. The present study is designed to fill some of these gaps in our knowledge, and especially to determine whether there are different climatic races within any of the species, and to compare the physio-



logical behavior of different species and genera.

The first step in the present investigation was to collect as many kinds of Lemnaceae as possible from a wide range of habitats in California. During October 1953, over one hundred strains were thus obtained. These were supplemented by strains kindly supplied by collectors from other parts of the United States, Central America, and Europe. Moreover, regular monthly visits are being made to approximately thirty stations in California in order to observe the seasonal development of different colonies in their natural habitats.

The plants collected in the wild are being maintained as crude cultures in pans kept in greenhouses at Stanford. A method has been developed for culturing individual clones free from microorganisms in sterile test tubes and flasks. Hutner's solution was used as a basic culture medium, since this proved to be superior to those of Hoagland and of Clark, media which have been most commonly used by other investigators of the Lemnaceae. Hutner's solution contains relatively high concentrations of micronutrients and also ethylenediamine tetraacetic acid (EDTA) as a chelating agent. To obtain cultures free from microorganisms, a single frond is placed in a solution of 0.5 per cent NaOCl for approximately one minute. Some 80 per cent of the plants so treated die, but among the survivors, some can be obtained free of other organisms. Some species seldom or never have survived in attempts made to date (*Lemna valdiviana*, *L. paucicostata*, *Wolffia*, *Wolffiella*), whereas others are disinfected more easily (*Spirodela polyrrhiza*, *Lemna minor*, *L. gibba*). Using this technique, approximately 70 pure clones of 7 species have been obtained.

Four cabinets 24 × 36 × 12 inches in size were constructed in which the cultures could be grown in Erlenmeyer flasks or test tubes under controlled conditions of light and temperature. The growth rate, dry weight, and area of the fronds produced were measured. The growth rate

( $K$ ) is constant for every race tested under the same conditions at the same season. This was measured as the difference between the logarithms of the number of fronds at the end ( $Nx$ ) and at the beginning ( $No$ ) of the experiment, divided by the number of intervening days ( $x$ ); or, expressed as an equation,

$$K = \frac{\log Nx - \log No}{x}.$$

The average dry weight per frond is determined by drying a certain number of selected fronds in a vacuum heater at 50° C for 4 hours and then weighing. For this purpose the largest fronds within a given culture are selected. The average dry weight per frond thus determined is constant for a clone cultivated under the same conditions. The average area of a frond is measured by projecting the images of a selected number of fronds on highly contrasting film with blue light shining underneath. After the film is developed, the images of the fronds appear to be white against a black background. The amount of light transmitted through the film can then be measured with a standardized photocell and the area of the fronds determined rapidly.

It was found essential to allow the strains to grow for about three weeks under the desired conditions of temperature and light before beginning measurements on growth rates; otherwise the values of the growth rate  $K$  were irregular, especially at lower temperatures. The temperatures in the four cabinets were maintained at 10° (or 14°), 20°, 26°, and 32° C. The light intensity was held at approximately 115 foot-candles, using fluorescent daylight-type lamps as a source.

When the cultures are grown at this relatively low light intensity and with the limited CO<sub>2</sub> supply that diffuses through the cotton-stoppered Erlenmeyer flasks from the surrounding air, the growth rate is greatly accelerated by adding 1 per cent sucrose to the nutrient medium. The curve in figure 1 shows the growth rate of a

strain of *Spirodela polyrrhiza* at 25° C in varying concentrations of sugar. This curve is based on data averaged from four replications.

With 1 per cent sucrose in the medium, growth rates increase as temperatures are increased, as shown in figure 2 for *Spirodela polyrrhiza*. Without sucrose the growth rate of this species is diminished at 32° C as compared with that at 26° C. When sugar is present, the average dry

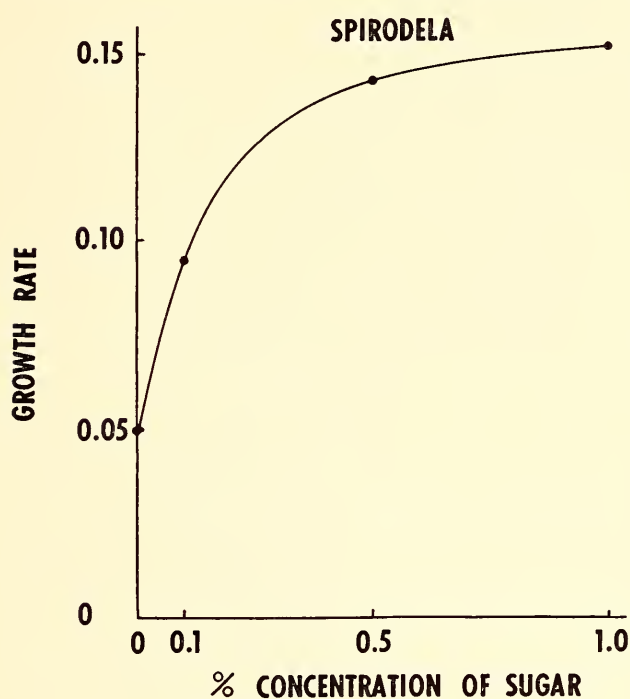


FIG. 1. Relation between sugar concentration and growth rate of *Spirodela polyrrhiza* from Centerville, California, at 25° C.

weight and the average area per frond increase at all temperatures.

The first experiments comparing the growth of five strains of three species at a series of temperatures show definite differences in growth rate. Examples are shown by the curves in figure 3. Included in the experiments were two populations of *Spirodela polyrrhiza* from the hot San Joaquin Valley of California: one of these was from near the Shawmut Grade in the lower Sierran foothills in Tuolumne County, and the other from near Centerville, Fresno County, approximately a hundred miles farther south. Both of these strains show remarkably identical rates of

growth at all four temperatures, so that they are represented by a single curve, the uppermost shown in figure 3. These two populations of a California interior valley race, however, have a consistently higher

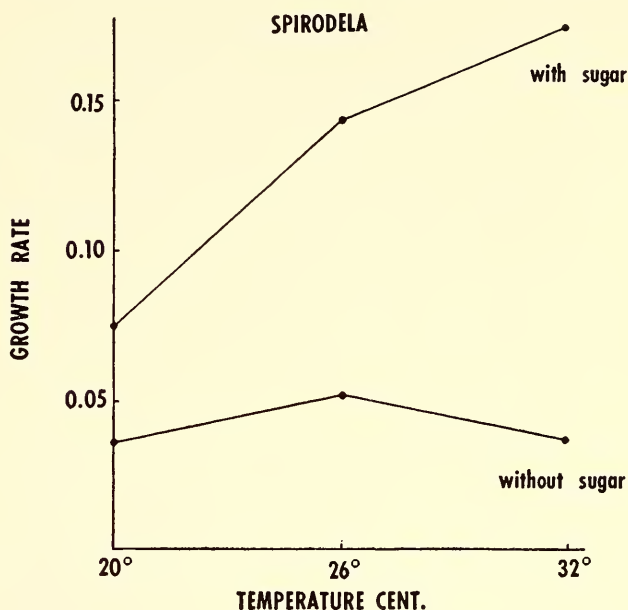


FIG. 2. Growth rate of *Spirodela polyrrhiza* from Centerville with and without sugar at three temperatures.

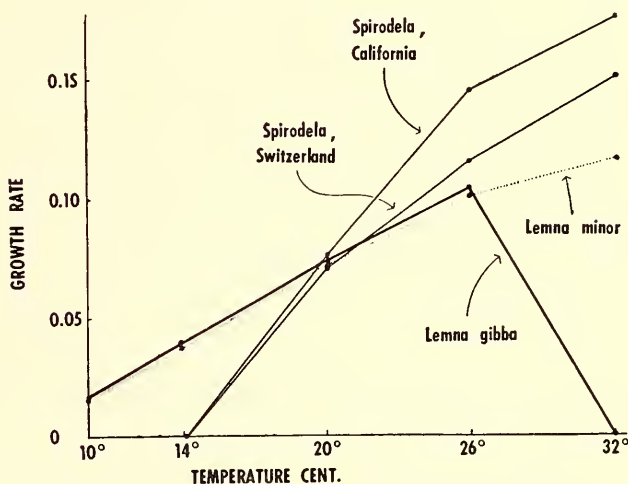


FIG. 3. Growth rate of five strains of Lemnaceae at different temperatures.

rate of growth at the warmer temperatures than a race of the same species from Lake Constance, Switzerland. At the cooler temperatures, the California and Swiss races show closely parallel rates of growth, and both fail to grow at all at 10° or at 14° C.

A marked difference in general growth pattern is evident between these three



strains of *Spirodela* and two species of *Lemna*. One form of *Lemna minor* from Harrington, New Jersey, and one form of *L. gibba* from Niles Canyon, near San Francisco Bay in California, both grow appreciably at 10° C. In the cultures of *Spirodela*, no growth takes place at this low temperature, and the plants develop into nonactive turion or resting stages. Between 10° and 26° C the two species of *Lemna* showed a remarkably parallel increase in growth rate, but at 32° C this particular form of *L. gibba* from the relatively cool California coast failed to grow and gradually disintegrated, whereas *L. minor* from New Jersey grew more rapidly than at 26° C.

It was observed that duplicate experiments with the same clones grown under the same conditions but at different seasons may not show quite the same growth behavior. During June, for example, the two strains of *Spirodela polyrrhiza* from California mentioned above grew about 10 per cent faster than during April. This suggests a seasonal periodicity similar to that reported by Pirson for root growth of *Lemna minor* at Marburg. If true periodicities exist in growth among the various strains of Lemnaceae, these periodicities need to be determined before valid comparisons can be made between growth rates of contrasting strains, races, species, and genera.

#### PHYSIOLOGY OF CLIMATIC RACES

HAROLD W. MILNER AND WILLIAM M. HIESEY

Work during the year was confined to the development of apparatus for the quantitative measurement of photosynthesis and respiration of plants under controlled conditions of temperature, gas composition, light, and relative humidity. The essential components and their principle of operation were described in the last Year Book (No. 52, pp. 176-178). Construction of the carbon dioxide analyzer and dehumidifier was completed, and these, with the oxygen analyzer, were incorporated in a

closed system and their operation was tested. The oxygen and carbon dioxide injectors and absorbers were designed and built. Work on the electronic controls for the entire apparatus is well advanced.

A preliminary arrangement of all the component parts into one system was made to permit testing of each unit and to observe the operating characteristics of the apparatus as a whole. It became apparent that a rearrangement of the parts was necessary to permit precise response of the apparatus and accuracy of measurement, and to eliminate the disturbing influence of external changes, particularly of temperature. It is anticipated that as soon as reassembly of the apparatus is completed, test runs on plant material can be started.

#### EVOLUTIONARY DIFFERENTIATION AT TROPICAL LATITUDES

JENS CLAUSEN

The visit to Brazil in the fall of 1953 presented a unique opportunity to observe different types of tropical vegetation over a range of about 30 degrees of latitude from the equator southward, and at altitudes ranging from sea level to more than 7000 feet. Latitudinal and altitudinal zonation in Brazilian vegetation appears to be similar to that found at temperate latitudes of North America, but the number of species in each plant community is far greater, a fact which should affect the evolution of genetic barriers. Brazil, which is considerably larger than the United States in area, has approximately 50,000 species of seed plants and ferns, or one-sixth of the total number of species of the world. Although the flora is so rich, its composition is entirely different from that of North America, for probably not more than a score of its numerous species are shared with the cool-temperate zone of the northern hemisphere. A few conspicuous examples of such species are the bracken fern, *Pteris aquilina*, a cudweed, *Gnaphalium purpureum*, and a few marsh plants such as *Typha* and *Potamogeton*. Al-

though plants in general populate almost every environmental niche on earth, not every plant family nor every plant order has evolved forms for all kinds of latitudes. In fact, strikingly few have. There are plants in all climates, but not all kinds of plants in all climates.

It is not yet known whether the patterns of evolution found in plant groups native to the temperate zone will also be found in groups characteristic of the tropics. This gap in our knowledge is a significant one, because evolutionarily tropical vegetation is probably older than vegetation of our present-day temperate zones. During the later Cretaceous and early Tertiary, when the seed plants rose to significance, the tropical type of vegetation had a far more extensive range than now, reaching far into regions that today are populated only by cold-temperate plants. Even today, the area of the earth covered by tropical vegetation is greater than that occupied by temperate-zone plants.

Some observations were made concerning the occurrence of periodicity at tropical latitudes, because such information has not previously been obtained. Seasonal periodicity is primarily a characteristic of plants native to climates that have seasonal periodic changes. In the equatorial zone, the climate is comparatively uniform the year round, and there would be no adaptive advantage to the plants of that zone in developing periodic changes following a regular annual cycle. Two of the most easily observed responses to seasonal changes are the deciduousness of leaves and the development of bud scales. Bud scales are special kinds of small, scaly leaves that protect the delicate growing point of the shoot. At temperate latitudes such a protection is essential, especially during seasons unfavorable for growth, whether they be too dry or too cold. On branches of trees from the temperate zone one can at all times of the year observe an alternation of the scars of the bud scales of previous years that have fallen off, and the long nodes separating the leaf scars and the

more closely congested scars of the bud scales. The development of bud scales is a major step in the adaptation of trees and perennials that enables them to populate less favorable regions. Observations made in the lowland forest of the Amazon region indicate that a few plants show periodicity in growth, but that the occurrence of specialized bud scales and deciduousness in annual cycles is rare. Deciduousness occurs in certain species, but apparently as a prelude to flowering, and not as a periodic rhythm directly connected with any season; moreover, there may be more than one period of flowering during a year. Most species show no indication at all of rhythm in growth.

On each side of the relatively narrow equatorial zone in all continents, there is a wide region having a true alternation between seasons, namely, a dry winter and a moist summer, the opposite of the Mediterranean-type climate. Vegetationally, these two symmetrical regions are occupied by savannas, in Brazil represented by the *cerrado* vegetation of low, scattered trees of many families. In area the equatorial savanna vegetations are probably the most extensive types of vegetation on the earth. In the *cerrado* region of Brazil the trees develop true bud scales in congested whorls as we know them in the temperate zone, and many of them lose their leaves during the dry but otherwise mild winter. Also, in the *cerrado* deciduousness is in rhythm with the annual seasons. This deciduous character is still facultative, however, because the trees of the *cerrado* lose their leaves only in unfavorable winters, and not always completely. They remain green during winters when the moisture is adequate. It is possible that the vegetation of the *cerrado* may furnish clues as to how the vegetation of past ages may have evolved forms able to cope with the general drying and cooling that appears to have followed after the humid, warm periods of the early Tertiary.

Another phenomenon at tropical latitudes is also of significance in the under-



standing of the dynamics of evolution. On the geologically old southeast Brazilian mountain massive, the tree line is abnormally low in altitude for the low latitudes,  $10^{\circ}$  to  $25^{\circ}$  south, and occurs at approximately 6000 feet, whereas in central California at latitude  $38^{\circ}$  north, trees can grow up to 11,000 feet altitude in the Sierra Nevada. In Brazil the *planalto* is a belt immediately above tree line. There the temperature and yearly precipitation are rather comparable with those of the coast of central California. One should, therefore, expect that redwood trees would be able to grow there. Nevertheless, the vegetation of the *planalto* reminds one of the Alpine fell fields. Some plants are of the low cushion type, similar to certain alpine of the Sierra Nevada in California or of the Swiss Alps, and others are more like the rosette shrubs above the tree line proper in the mountains of equatorial Africa. Apparently none of the numerous species of trees in the cloud forests below the *planalto* in Brazil have evolved species or races that are able to tolerate occasional temperatures only a few degrees below freezing.

The reason for the low tree line at tropical latitudes in Brazil is probably to be sought in the character of the germ plasm of the vegetation at the low altitudes there. The genera, families, and even orders of plants that form the vegetation of the forests below tree line have during our present geologic period been unable to migrate beyond the warm-temperate zones, and there is indication that some of them become damaged by cold even before the temperatures drop to the freezing point. One of the tree species most tolerant to cold in the tropical mountain forest is *Drimys Winteri*, of the Winteraceae of the order Magnoliales. In the mountain forests below the *planalto* this species forms large trees, but it is barely able to survive in the relatively mild climate of the *planalto*, where scattered, dwarfed, weather-beaten specimens cling to large rocks.

In contrast, the extreme tolerance to cold of the conifers and other plants in the high altitudes of the Sierra Nevada in North America is amazing. In early September, the trees and many herbaceous flowering alpine at our Timberline station can emerge from periods of snow and freezing weather as low as  $-13^{\circ}$  C with buds and flowers completely intact, and during the period of dormancy in a winter nine months long they tolerate much lower temperatures. Apparently there are large segments of the plant kingdom, including orders such as the Myrtales which are prevalent mostly as trees at tropical latitudes, that, during their long evolutionary history, have been unable to develop truly cold-tolerant plants like the conifers and the birches of northern latitudes.

#### NUCLEIC ACID AND PROTEIN CONTENT OF ISOLATED ROOT-TIP NUCLEI

PAUL GRUN

A study of the nucleic acid and protein content of interphase root-tip nuclei of *Tradescantia paludosa* (Year Book No. 52, pp. 178-179) was carried out during a year spent in Stockholm, Sweden, at the laboratory of Professor T. O. Caspersson, and the analysis of the data was completed at the Institution's laboratory at Stanford. Analysis of ultraviolet absorption spectra of the nuclei was used to determine the quantity of nucleic acids—deoxyribonucleic acid (DNA) plus ribonucleic acid (RNA)—and protein, as indicated by the amino acids tyrosine and tryptophane, present per square micron of nuclear surface. By multiplying these figures by the area of the nuclear surface, the relative total nucleic acid and protein content of each nucleus could be calculated.

The data were analyzed in order to answer two questions: (1) When in the mitotic cycle does the nucleic acid and protein content of nuclei increase? (2) Is there any consistent quantitative difference in nucleic acid and protein content between nuclei from cells in the active divi-

sion cycle and those that have ceased division?

As an approach to the first question, correlation coefficients were determined to measure the relation between total protein and nucleic acid content and nuclear area, in order to evaluate the change in the quantity of the constituents as nuclei grow during interphase. These coefficients indicated that the pattern of increase of nucleic acid in the nuclei contrasts sharply with that of the proteins. As the nuclei grew there was a highly significant increase in the total quantity of nucleic acids present. This pattern applied to nuclei of the "differentiated area," with an  $r$  value of  $-0.67$ , as well as to those of the "mitotic area" ( $r = -0.74$ ). It is not at present known whether this increase is predominantly in RNA or DNA. The quantity of nucleic acid per nucleus extended through a continuous series from ca.  $12 \times 10^{-10}$  mg to ca.  $836 \times 10^{-10}$  mg. The nucleus richest in nucleic acid therefore contained approximately 70 times as much as the nucleus which was poorest in nucleic acid. This fact taken with the commonly observed constancy of DNA in plant and animal cell nuclei suggests that the nucleic acid measured was chiefly RNA.

There was a highly significant negative correlation between nuclear area and quantity of protein present per square micron of nuclear surface. For nuclei of the differentiated area,  $r = -0.54$ , whereas for the mitotic-area nuclei  $r = -0.57$ . This indicated that as the nuclei grew there was no corresponding increase in total protein, but rather that the quantity present was diluted through the increasing nuclear volume. On the average, therefore, large nuclei contained no more total protein than did small nuclei. These results suggest that increase in protein does not occur during interphase, but during some stage of the actual cell division.

To answer the second question, nuclei of the mitotic and differentiated areas were compared with respect to nuclear area, total nucleic acid content, total protein

content, and total nucleolar areas. The nuclei of the mitotic area were very significantly smaller ( $t = 5.00$ ) than were those of the differentiated area. There was an overlap in sizes, but the smallest nuclei, representing probably those that had just entered interphase after a cell division, were found only in the group from the mitotic area. Since the quantity of nucleic acids was correlated with nuclear size, the nuclei of the mitotic area also showed a very significantly lower nucleic acid content than did those of the differentiated area (chi square against equality = 15.02). There was no significant difference between the total quantity of protein found in differentiated and in mitotic nuclei, the  $t$  value of the difference being 1.70. These findings suggest that lack of cell division of nuclei from the differentiated area does not result from a failure of nucleic acids and proteins to increase. The larger size and higher nucleic acid content of the differentiated nuclei might more easily be interpreted as a result of, than as a cause of, failure of cell division.

Earlier studies by Professor Caspersson have shown that one generally clear-cut difference between nuclei of actively dividing or metabolizing cells and those of less active cells is to be found in the sizes of the nuclei and the globular bodies (nucleoli) within them. The nucleoli of active cells are generally larger than those of inactive cells. It has been demonstrated, using several different techniques, that the nucleoli are rich in RNA, and this has led to the suggestion that they may be the seat of most of the nuclear RNA.

Camera lucida drawings of *Tradescantia* root-tip nuclei and their nucleoli were made at known magnifications, and areas were measured using a planimeter. The average nucleolar area of the mitotic nuclei was found to be substantially larger than that of differentiated nuclei,  $t$  of the difference being 19.3. As indicated above, the large range in nucleic acid content of these nuclei suggests that the chief varia-



tion is in RNA, and therefore that the large differentiated nuclei have a high RNA content. If these nuclei are rich in

RNA, however, it is not nucleolar RNA, for the nucleoli of the differentiated-area nuclei are relatively few and small.

## BIOCHEMICAL INVESTIGATIONS

### REVERSIBLE CHANGES IN THE ABSORPTION SPECTRA OF RHODOSPIRILLUM AND CHLORELLA ON IRRADIATION

L. N. M. DUYSSENS

Methods for measuring changes in absorption spectra have been of prime importance in the elucidation of enzymatic reactions, particularly of the cytochromes in nonphotosynthetic organisms. One of the main difficulties in the study of photosynthesis by the common biochemical methods has been that photosynthesis stops when the cells are broken. Therefore optical methods are even more important for photosynthesizing organisms, since they can be applied to intact cells. Nonphotosynthetic organisms generally contain only small amounts of other screening pigments. In photosynthesizing organisms, however, there are greater technical difficulties in the measurement of these spectral changes, due to the strong absorption by the photosynthetic pigments and to danger of interference by the intense irradiation needed to produce the changes with the light used for measuring absorption. These difficulties were overcome by employing a sensitive and selective apparatus which detected only the modulated light from a measuring and compensating beam. Second-order effects of irradiating light on the detector were minimized by complementary filtering of irradiating and measuring beams, or by irradiating the suspension in a separate chamber before it was pumped rapidly through the absorption vessel (Year Book No. 52, 1952-1953, p. 158).

A change in the absorption spectrum of bacteriochlorophyll in purple bacteria (*Rhodospirillum rubrum* strain 1) was observed upon irradiation only if the suspension medium contained an oxidizing agent, such as oxygen or potassium ferri-

cyanide. In reducing media such as the growth medium (1 per cent peptone) or weak hydrosulfite solution, only a small change, or none at all, was observed.

It was thought that irradiation changed bacteriochlorophyll to an oxidized state. In the supposed presence of some bacterial substance XH, which had been reduced by cell metabolism or by a reductant in the medium, the oxidized bacteriochlorophyll would not accumulate, but would react rapidly with XH and be returned to its original state. If, on the other hand, the substance was present in its oxidized state, X, oxidized bacteriochlorophyll would accumulate and a change in the absorption spectrum would be observable. The substance X should be readily reducible by hydrosulfite. One of the substances of possible significance, which has this property, is cytochrome *c*. Furthermore, cytochrome *c* itself shows a change in its absorption spectrum in the visible region, when it passes from its reduced to its oxidized state.

In order to measure changes in absorption spectra in the visible region, we modified the apparatus used before. Monochromatic light was split into two beams by means of a rotating sector disk driven by a synchronous motor. Mirrors formed the solid sectors of the disk, alternating with open sectors. The measuring beam, interrupted 60 times per second by the mirrors, caused a deflection of a recorder opposite to the deflection caused by the compensating beam, which was reflected by the mirrors. Both beams fell on a photomultiplier, which was connected to an AC amplifier. After amplification, the AC signal was rectified by a phase-sensitive demodulator, employing a double diode and a reference voltage from the AC line. The rectified voltage was fed into a Brown

recorder by way of balanced cathode followers. The organisms were in an absorption vessel, which was placed in the measuring beam.

Changes in the visible region of the absorption spectrum of a suspension of photosynthesizing organisms were brought about by irradiation of the organisms with red and infrared light. After this light was shut off, the original absorption spectrum returned usually within a few seconds.

This reversible change of spectrum was observed in purple bacteria when they were actively photosynthesizing. The suspension was irradiated with light of moderate intensity, not much higher than that necessary to bring about saturation of photosynthesis. The difference between the absorption spectra of the bacteria in the light and in the dark is plotted in figure 4, which also shows the difference spectrum of oxidized and reduced cytochrome *c*. Comparison of these spectra shows that a cytochrome-like pigment was indeed oxidized in the light and reduced in the dark. The change in bacterial pigment seems similar to, but, because of a small wave-length shift in the maxima, not identical with cytochrome *c* in spectral properties.

In the presence of oxygen and in the absence of substrate, the difference between the spectra in light and in darkness was, at moderate intensity of irradiation, completely different from that shown in figure 4. It is possible that the difference spectrum in the presence of oxygen is due, at least partly, to oxidized bacteriochlorophyll.

When most of the oxygen was removed and the bacteria were left for some time in the dark in a stoppered absorption vessel, which was filled to the top, an increase in absorption occurred at 430 m $\mu$ . This increase indicated the reduction of the cytochrome by metabolic dark processes. After, and only after, this increase had taken place, the spectral changes brought about by moderate light were those given in figure 4, showing oxidation of the cytochrome.

Before the change in the dark had taken place, irradiation produced an increase in absorption at 430 m $\mu$ .

An attempt was made to find out what happens when in the absence of oxygen the bacteria are illuminated with an intensity higher than is necessary to oxidize all cytochrome. The bacteria were suspended in oxygen-free 1 per cent peptone solution, their growth medium. As was mentioned before, the difference spectrum at moderate light intensity was that of the cytochrome. If the irradiation was far above saturating intensity, the change in absorption occurring on onset of irradiation

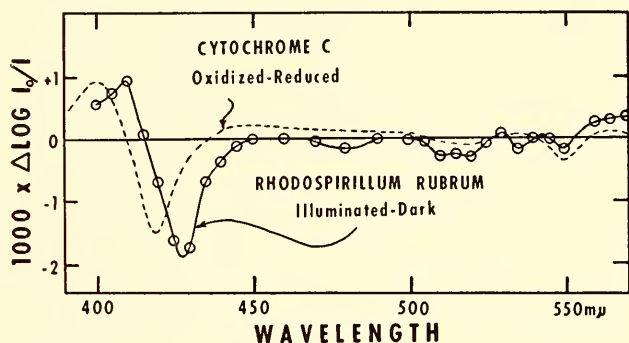


FIG. 4. Difference between absorption spectra in light and in darkness of the purple bacterium *Rhodospirillum rubrum* strain 1, compared with the difference between oxidized and reduced cytochrome *c*.

tion had the same direction as when the cytochrome was oxidized. Within a few seconds of irradiation, however, this spectrum was overlaid by a different spectrum, which, as measurements at a few wave lengths suggested, was the same as that occurring in a suspension lacking substrate but containing oxygen.

It seemed that the strong irradiation caused first the oxidation of the cytochrome and then the accumulation of another substance, which we have suggested to be oxidized bacteriochlorophyll. These findings fitted very well with what was known about cytochrome *c* and about the over-all reactions of photosynthesis and of respiration in *Rhodospirillum rubrum* strain 1.

In the organisms studied, cytochrome *c*, or a similar enzyme, probably functions as



an intermediate in oxidations by molecular oxygen. This oxidized cytochrome was reduced by enzymes, which in their turn were reduced directly or indirectly by the substrate. The reduced cytochrome was oxidized by an enzyme that was either directly or indirectly oxidized by oxygen. It was found that light partially suppressed the respiration in *Rhodospirillum rubrum* strain 1, but that the substrate was used up at the same rate in the light as in the dark. This finding indicated that the substrate was oxidized both in respiration and in photosynthesis by a common mechanism. Our experiments strongly suggest that, at least in purple bacteria, not only the respiratory oxidation but also the photosynthetic oxidation is mediated by a cytochrome-like pigment. The photosynthetic oxidation of the substrate by a cytochrome may, like the respiratory oxidation, result in the generation of energy-rich phosphate bonds, in the form of ATP, which seems to be needed for providing additional energy in photosynthetic carbon dioxide reduction.

Using the same apparatus, we found that changes in absorption spectrum occur also in the green alga *Chlorella* and in higher plants. The difference spectrum for *Chlorella* and for a few green plants studied was unlike that observed for purple bacteria. The spectrum for *Chlorella* is shown in figure 5. This difference spectrum has a maximum at 510 m $\mu$ , indicating an increase in absorption on irradiation, and negative maxima at 480 and 420 m $\mu$ , indicating decreases in absorption. The maxima at 510 and 480 m $\mu$  were also observed in the green plants, for which the spectrum was not measured below 480 m $\mu$ . We have not yet been able to identify the substance causing the spectral changes in the region 460–540 m $\mu$ . By analogy with purple bacteria, the maximum of the *Chlorella* spectrum at 420 m $\mu$  may be due to oxidation of a cytochrome pigment. We think here of cytochrome *f*, the only cytochrome known with certainty to occur in chloroplasts.

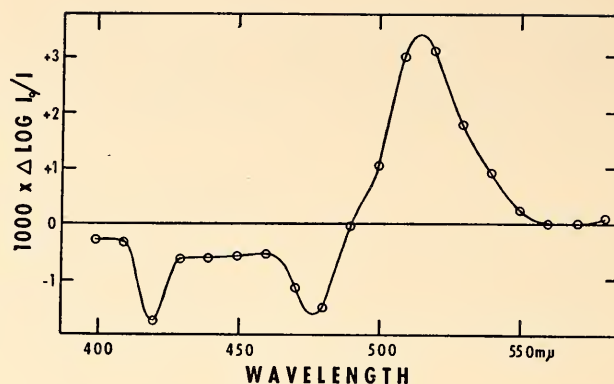


FIG. 5. Difference between absorption spectra in light and in darkness of the green alga *Chlorella*.

#### ABSORPTION SPECTRA OF CHLOROPHYLLS

JAMES H. C. SMITH AND ALLEN BENITEZ

The absorption coefficients at wavelengths throughout the visible spectrum have been determined for chlorophylls *a*, *b*, *c*, *d*, and bacteriochlorophyll in our laboratory this year. This fundamental physical property is useful both for determining the chlorophylls quantitatively and for interpreting their relation to photosynthesis.

Although quantitative absorption spectra of chlorophylls *a* and *b* had been published previously, such discrepancies existed between the published values as to warrant their redetermination, especially by a different method, which we have used. With the increased interest in the photosynthesis of organisms containing chlorophylls other than *a* and *b*, it has become desirable to know the quantitative absorption curves of these pigments also. A full report on these measurements has been incorporated into a chapter entitled "Chlorophylls: Analysis in Plant Materials" which will appear in the forthcoming book *Modern Methods of Plant Analysis*. A résumé of our measurements is given here.

The chlorophylls were extracted from their plant sources and purified by chromatographic procedures: chlorophylls *a* and *b* were obtained from grape leaves; chlorophyll *c* from the brown alga *Nereocystis Luetkeana*; chlorophyll *d* from the

red alga *Gigartina Agardhii*; and bacteriochlorophyll from the purple bacterium *Rhodospirillum rubrum*.

Each chlorophyll was purified by the procedures of chromatography, using confectioner's sugar as adsorbent and a series of organic solvents as chromatographic developers, until it was the only colored substance present in the ether solution of which the absorption spectrum was measured.

A quantitative absorption curve is obtained by plotting specific absorption coefficients against wave lengths. The specific absorption coefficient,  $\alpha$ , relates optical density,  $D$ , of the solution to the concentration of pigment in grams per liter,  $C$ , and the depth of the solution in centimeters,  $d$ , according to the equation  $D = \alpha d C = \log_{10}(I_0/I)$ . The quotient,  $I_0/I$ , is the ratio of the intensities transmitted by equal depths of pure solvent and solution.

The concentration of the chlorophyll required to compute the specific absorption coefficient was determined from the magnesium content of the solution used for spectrophotometric measurement. The magnesium content per liter of solution divided by the magnesium content of the chlorophyll gave the concentration of chlorophyll in grams per liter. The magnesium analysis was made by a spectrophotometric method capable of determining from 0.050 to 0.100 mg of magnesium.

The absorption curves of the different chlorophylls obtained by the methods outlined are shown in figure 6 by the solid lines.

Closely related to the chlorophylls are their magnesium-free derivatives, the pheophytins. Frequently, it is advantageous to know the absorption spectra of these derivatives for analytical purposes. Consequently, the pheophytin corresponding to each chlorophyll has been prepared by treatment of the chlorophyll with acid, and its absorption spectrum has been determined. Since the conversion is quantitative, the pheophytin concentration is easily reckoned when a chlorophyll solution of

known concentration is transformed. From this concentration and the spectrophotometric measurements, the specific absorption coefficients of the pheophytin at various wave lengths are calculated. Plots of these coefficients against wave length for the different pheophytins are given in figure 6 by the dashed lines.

At present, the most commonly accepted values for the spectroscopic constants of chlorophylls  $a$  and  $b$  and pheophytins  $a$  and  $b$  in ether are those of Zscheile and Comar (1941). Comparison of our values with theirs for the absorption coefficients of chlorophyll  $a$  in ether shows good agreement between the two. Our measurements indicate, however, that their values for the absorption coefficients of chlorophyll  $b$  and of pheophytins  $a$  and  $b$  are about 10 per cent too low. Also, our values for the wave lengths of the absorption maxima are mostly 1 or 2 m $\mu$  greater than theirs. Plots of our values for the specific absorption coefficients of these four pigments in ether against wave length are given in sections A and B of figure 6.

No reliable determinations of the absorption coefficients of chlorophyll  $c$  or of pheophytin  $c$  have been obtained. The reasons for this are as follows:

Preparations having constant and reproducible spectroscopic properties are difficult to obtain. Various similar chromatographic fractions of the pigment exhibit somewhat different absorption spectra, as the solid and dotted lines of section C of the figure demonstrate.

Also, the molecular weight of chlorophyll  $c$  is unknown. This makes it impossible to determine the true specific absorption coefficients from magnesium analysis of the solution used for spectrophotometric measurement. For this reason, the legend of the left-hand ordinate is put in quotation marks. The values on this ordinate have been calculated from a magnesium determination of a chlorophyll- $c$  solution, whose absorption curve is plotted by the solid line, and are based on the assumption that chlorophyll  $c$  has the same



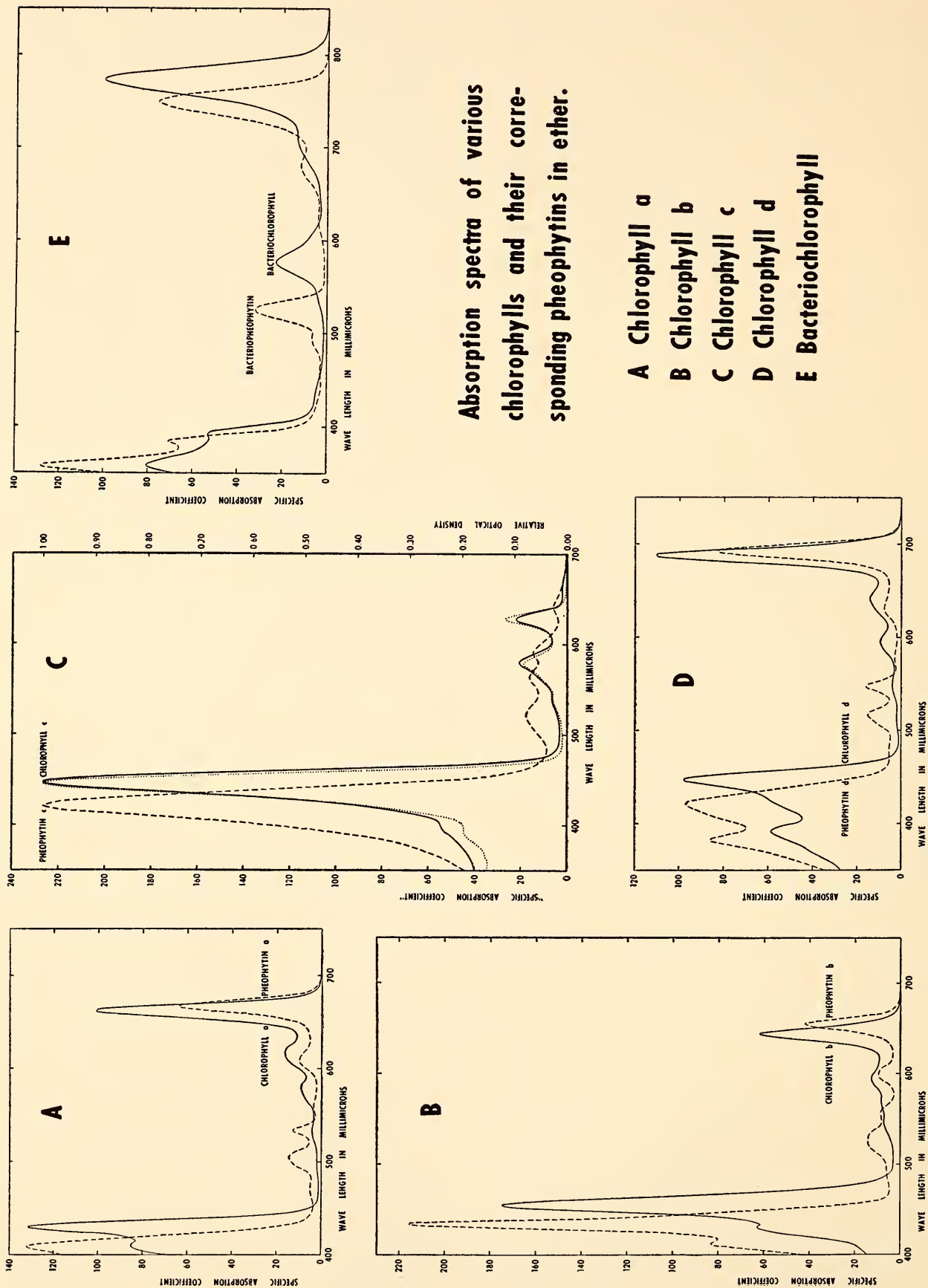


Fig. 6. Comparison of the absorption spectra of various chlorophylls and their corresponding pheophytins in ether solution

molecular weight as chlorophyll *a*, namely, 893.5. This assumed molecular weight could be 30 per cent too great, because chemical tests indicate chlorophyll *c* to be phytol-free.

For the chlorophyll-*c* fraction represented by the dotted line, only relative optical density values are available. In order to compare the absorption curve of this chlorophyll-*c* fraction with that represented by the solid line, the short-wave-length maxima of the two curves have been made equal in height.

Chlorophyll *c* is extracted from ether solution by 12 per cent hydrochloric acid but not by dipotassium hydrogen phosphate or potassium bicarbonate. This behavior suggests that the phytol group present in most chlorophylls has been replaced by a smaller alkyl group.

An ether solution of chlorophyll *c* gives a transient reddish-brown color when it is treated with 30 per cent methanolic potassium hydroxide. This indicates the presence of a cyclopentanone ring (ring V) in chlorophyll *c*.

Pheophytin *c* is strongly adsorbed from ether solution on the walls of containing vessels, even on the walls of cells used for measuring its absorption spectrum. This fact caused uncertainty regarding its concentration in solution. It was therefore impossible to get trustworthy values for its specific absorption coefficients, and consequently only relative optical densities at the different wave lengths were obtained. These relative values of the optical densities (right-hand ordinate, section C, fig. 6) are plotted against wave length by the dashed line. The height of the short-wave-length maximum of this curve was arbitrarily set equal to that of chlorophyll *c* (solid line).

The absorption curves of chlorophyll *d* (solid line) and pheophytin *d* (dashed line) are drawn in section D of figure 6. The specific absorption coefficients of chlorophyll *d* in ether were obtained from magnesium analysis of the solution used for spectrophotometric measurements and

calculations based on an assumed molecular weight of 893.5 for the pigment. This assumed molecular weight is probably not greatly in error, since concentrations of hydrochloric acid greater than 22 per cent were required to extract the pigment from its ether solution. From this, it may be inferred that chlorophyll *d* has a phytol group.

A transient red-brown coloration is produced in an ether solution of chlorophyll *d* when it is treated with 30 per cent potassium hydroxide in methanol. The assumption is, therefore, that chlorophyll *d* contains the cyclopentanone ring characteristic of other chlorophylls.

The specific absorption coefficients of pheophytin *d* are subject to whatever uncertainties are inherent in the coefficients obtained for chlorophyll *d*, inasmuch as the concentration was calculated on the basis of an assumed molecular weight of 871.2, the molecular weight of pheophytin *a*. The similarity in the shapes of the absorption curves of pheophytin *d* and pheophytin *a* is worthy of note.

The absolute absorption curves of bacteriochlorophyll and bacteriopheophytin in ether solution which we have obtained are presented in section E of figure 6.

No quantitative absorption curve of bacteriochlorophyll has been published previously. The only comparison with previous work that can be made is with the values of the specific absorption coefficients at the absorption maxima obtained indirectly by Weigl in 1953. For the wave lengths of the absorption maxima, his values and ours agree very well, but the agreement is not uniformly good among the values for the absorption coefficients.

There is no absorption curve of bacteriopheophytin in ether available for comparison with our curve. The wave-length positions of the absorption maxima determined by Weigl and by us are in excellent agreement. The specific absorption coefficients at the maxima obtained through indirect means by Weigl are in only fair agreement with our own.



French (1940) has measured the absolute absorption spectrum of bacteriopheophytin in chloroform. For comparative purposes, we transferred some of our pigment to this solvent and determined its absorption spectrum. Except at the long-wave-length maximum, there was reasonably good agreement between the two measurements both as to wave lengths and as to specific absorption coefficients of the maxima.

The spectroscopic constants of protochlorophyll have been given by Smith and Koski (Year Book No. 47, 1947-1948, p. 94).

At present, bacterioviridin, the chlorophyll of the green sulfur bacteria, is the only naturally occurring chlorophyll known with certainty to exist whose absorption spectrum has not been measured quantitatively.

#### FLUORESCENCE SPECTRA OF CHLOROPHYLLS AND PHEOPHYTINS

J. H. C. SMITH, C. S. FRENCH, H. I. VIRGIN,  
AND A. BENITEZ

As was stated in the previous section, solutions of various chlorophylls and their pheophytins have been prepared and their absolute absorption spectra measured. Also, the fluorescence spectra of these pigments have been obtained. These fluorescence spectrum measurements are very useful for chlorophyll analysis for several reasons:

Fluorescence is an extremely sensitive test for small concentrations of chlorophyll. This makes the detection of small quantities of chlorophyllous impurities possible. For example, it has been estimated that chlorophyll *a* can be determined at concentrations of 1 microgram per liter.

The shape of the fluorescence spectrum curve of a pure fluorescent substance is relatively insensitive to the influence of small quantities of other nonfluorescent substances and is independent of the wave length used for exciting the fluorescence. Deviations observed in the shapes of fluo-

rescence curves, therefore, indicate contamination. This is especially true when the shapes of the curves are altered by changing the wave lengths of excitation.

From the deviations observed in the shape of a fluorescence curve, evidence concerning the nature of the contaminant may be obtained. This is of considerable help in pointing the way to further purification of the chlorophylls.

Use has been made of these facts in controlling the separation of the chlorophylls and in determining the purity of the samples prepared.

An example of the use of different exciting wave lengths to determine whether a fluorescence band was the property of a given pigment or was due to an impurity is the case of bacteriochlorophyll. Alcoholic solutions of this pigment had been reported by Vermeulen, Wassink, and Reman (1937) to have fluorescence maxima at 695 and 805 m $\mu$ . This presented an anomaly, since the long-wave-length absorption band of this pigment lay at longer wave lengths than its 695-m $\mu$  fluorescence band. In our laboratory it was found that when a chromatographically purified sample of this pigment dissolved in ether was irradiated with wave length 436 m $\mu$ , a red fluorescence maximum appeared at 687 m $\mu$  which was more prominent than the infrared fluorescence, whereas when the pigment was irradiated with wave length 405 m $\mu$  the infrared was more prominent. In a separate experiment, the fluorescence at 687 m $\mu$  was demonstrated to be characteristic of a contaminating green pigment which was difficult to eliminate. Consequently, it is the infrared fluorescence which belongs to bacteriochlorophyll, and not that at 687 m $\mu$ . Thus the anomaly in the fluorescence of bacteriochlorophyll has been resolved.

The fluorescence curves of chlorophylls *a* and *b* in ether solution are shown in figure 7. In this figure the curves obtained in our laboratory are compared with those recalculated from the data of Zscheile and Harris (1943). The curves for chlorophyll

*b* are in excellent agreement. The curve of Zscheile and Harris for chlorophyll *a* lies at about 3.5 mμ shorter wave length than our curve. A similar difference exists between the absorption curves for chlorophyll *a* obtained in the two laboratories. The cause of this difference for both fluorescence and absorption curves is not immediately apparent.

In general, the fluorescence curves for the various chlorophylls and pheophytins have the same shapes as those shown for chlorophylls *a* and *b* in figure 7. From the curves obtained in our laboratory, we

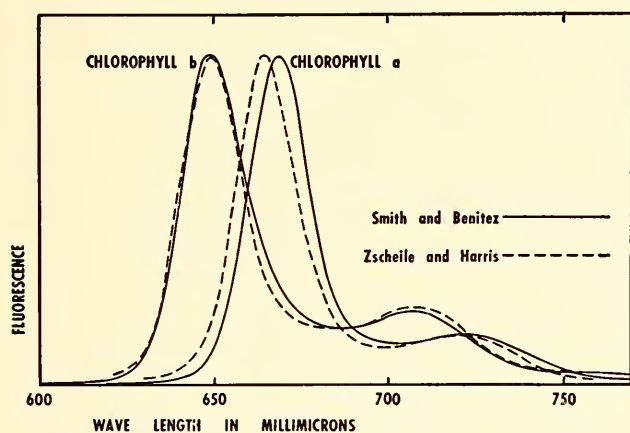


FIG. 7. Comparison of the fluorescence spectrum curves of chlorophylls *a* and *b* in ether obtained by Zscheile and Harris and in this laboratory.

have determined the wave lengths at the maxima of the major and minor fluorescence bands. These values are presented in table 3. Two points should be noted concerning the values in the table: first, the fluorescence bands of pheophytin *c* are very distinct, widely separated, and of comparable height; second, the minor fluorescence band of pheophytin *d* is very indistinct and forms only a shelf on the long-wave-length side of the major fluorescence band of this pigment.

#### THE DISTORTION OF FLUORESCENCE SPECTRA BY LIGHT SCATTERING

HEMMING I. VIRGIN

The fluorescence spectra of chloroplast suspensions have a characteristic shape with a rather high peak at about 685 mμ

and a low peak at 735 mμ. In the case of chlorophyll solutions, the shapes of the curves are similar but the peaks are shifted to shorter wave lengths, their exact position depending on the solvent used. If no reabsorption takes place, the ratio in height of the two peaks is about 4:1 or more.

The fluorescence spectrum of a living tissue, e.g., a leaf or some other green part of a plant, also shows two peaks, but the

TABLE 3

WAVE-LENGTH POSITIONS OF MAXIMA OF MAJOR AND MINOR FLUORESCENCE BANDS OF VARIOUS CHLOROPHYLLS AND PHEOPHYTINS IN SOLUTION

| PIGMENT              | SOLVENT                   | POSITIONS OF MAXIMA (IN Mμ) |       |
|----------------------|---------------------------|-----------------------------|-------|
|                      |                           | Major                       | Minor |
| Chlorophyll <i>a</i> | ....Ether                 | 668                         | 723   |
| Chlorophyll <i>b</i> | ....Ether                 | 649                         | 708   |
| Pheophytin <i>a</i>  | ....Ether                 | 672.5                       | ≈715  |
| Pheophytin <i>a</i>  | ....Methanol+<br>4% ether | 672                         | 720   |
| Pheophytin <i>b</i>  | ....Ether                 | 657                         | 707   |
| Pheophytin <i>b</i>  | ....Methanol+<br>4% ether | 657.5                       | 707   |
| Chlorophyll <i>c</i> | ....Ether                 | 629                         | ≈690  |
| Pheophytin <i>c</i>  | ....Ether                 | 649                         | 719   |
| Chlorophyll <i>d</i> | ....Ether                 | 696                         | ≈752  |
| Pheophytin <i>d</i>  | ....Ether                 | 701                         | Shelf |
| Protochlorophyll     | ..Acetone                 | 627                         | ≈685  |

ratio of height differs considerably from that for chlorophyll solutions. Moreover, the variations in relative height are great, and sometimes the peak at 735 mμ predominates over the peak at 685 mμ.

It has been speculated that this difference between living tissue and pigment extracts may be due in part to additional pigments. A study of the discrepancies between the two systems shows that the peculiar shape of the fluorescence curve in vivo is mainly a result of light scattering and reabsorption in the plant.

The first clue was the observation that in some cases the distortion was greater in the spectrum of a pale leaf containing a rather low concentration of chlorophyll



than in the spectrum of a dark green leaf. This pointed toward the fact that the concentration of chlorophyll itself could not be the cause for the distortion of the spectrum. It was also found that if the leaf had been subjected to pressure resulting in a partial infiltration, the distortion of the spectrum was decreased. Infiltration with water by means of vacuum treatment also changed the shape of the spectral curve toward that of a chlorophyll solution. The final proof that light scattering is the main cause for the distortion was given when it was shown that a piece of uncompressed glass wool moistened with a suspension of chloroplasts gave the same kind of spectrum as a living leaf rich in intercellular spaces.

Because of the extremely complicated optical system formed by the cell walls, the cell contents, and the intercellular spaces, it is not possible to approach this problem of light scattering quantitatively. A possible explanation of the way in which it may bring about the difference between spectral curves obtained in pigment solution and in the living tissue has, however, been presented, and will be published in *Physiologia plantarum*.

In connection with this investigation a study was also made of the spectra during the rapid change in the fluorescence yield occurring in the first 5 to 10 minutes of illumination following a dark period. This change consists of rapid increase in fluorescence completed within a few seconds. The value then obtained will decrease slowly until a steady state is reached, when the intensity of the fluorescence may be as low as one-third of the maximum value.

The materials used were leaves of *Arbutus Menziesii* and *Montia perfoliata*, thalli of the alga *Ulva lactuca*, and suspensions of chloroplasts from *Spinacia oleracea*. The leaves and the thalli to be investigated were kept in darkness for one day prior to the measurement. They were then illuminated for a short period with light of 436 m $\mu$ , giving a high fluorescence yield. After a few seconds the maximum fluores-

cence was reached (measured at 685 m $\mu$ ). The light was shut off and the object was left in complete darkness for 8 to 10 minutes, when the illumination was repeated. After this time in darkness, maximum fluorescence was again obtained. When five consecutive measurements gave equal values, the whole spectrum was measured by changing the wave-length setting of the analyzing monochromator 10 m $\mu$  at a time. The fluorescence spectrum for the steady state was taken after the object had been continuously illuminated for 10 minutes with the incident light used for exciting fluorescence.

The results of these measurements showed that the decrease in fluorescence yield does not take place proportionally for all wave lengths. The fluorescence around 685 m $\mu$  decreases more than that around 735 m $\mu$ . This can be seen by reducing the curve for the initial fluorescence so that its height at 685 m $\mu$  equals that for the observed steady state at the same wave length. Leaves from many different species, in addition to those mentioned here, have been investigated, and the same type of disproportionality has always been found.

If now the same experiments are performed with infiltrated leaves, the difference between observed and calculated spectra is much smaller. With thalli of *Ulva*, where there are no air-filled intercellular spaces, the spectrum found and the calculated steady state show complete congruence.

The conclusion can thus be drawn that some changes occur in the tissues, during the time it takes for the fluorescence to reach the steady state, which are large enough to cause a change in the air volume or in some other optical property of the intercellular spaces. The change in the spectral curves during this period of intensity change is of the same nature as the change which would be obtained if the intercellular spaces were increased.

Freshly prepared suspensions of chloroplasts which show ability to liberate oxygen

from water under the influence of strong light also give the afore-mentioned changes in the fluorescence yield at the beginning of illumination. Such suspensions, kept in darkness, were passed through a channel  $0.85 \times 0.06 \times 10$  mm in size at different rates of flow. The channel was exposed to the exciting light. At any constant flow rate the fluorescence will remain constant, but its intensity will be dependent on the flow rate. A very rapid flow gives the fluorescence at the very beginning of the fluorescence time curve, whereas a low flow rate gives the average intensity for the interval of time in question. In this way it was possible in a short time to get the whole fluorescence spectrum at different intensities of fluorescence. If the curves thus obtained are proportionally reduced to match the same height at a particular wave length, they are all identical, i.e., the change in the intensity of the fluorescence during the initial rise to the maximum intensity is the same over the whole wavelength range. This fact is consistent with the idea that previously mentioned irregularities in the fluorescence spectrum of normal leaves are due to scattering of the light in the mesophyll layers.

An implication of these experiments is that infiltration of plant tissues before measuring the fluorescence spectrum may prove to be a useful method for increasing the possibility of using the fluorescence data quantitatively, since most of the variations due to differences in intercellular spaces can be eliminated.

#### STUDIES OF THE CONVERSION OF PROTOCHLOROPHYLL TO CHLOROPHYLL IN FLASHING LIGHT BY MEANS OF FLUORESCENCE SPECTROPHOTOMETRY

HEMMING I. VIRGIN

The kinetics of the photochemical reaction whereby protochlorophyll is transformed to chlorophyll in the living plant has been the object of earlier studies at this laboratory. As has been shown by Dr. J. H. C. Smith and Mr. Allen Benitez, this

reaction has a low temperature coefficient and follows the time course of a second-order reaction. Their method of following the reaction was based on spectrophotometric absorption measurements of the extracted pigments after different periods of exposure to continuous illumination.

The fact that the reaction follows the second-order law suggests the possibility that this pigment conversion may also involve a light-independent chemical reaction. An answer to this question can be obtained by studying the rate of pigment conversion under the influence of light flashes of short duration separated by considerably longer dark periods, as compared with the conversion obtained after illumination with the same amount of light energy given continuously. If a chemical reaction is involved, a higher yield might be found after illumination with intermittent light than that obtained under continuous illumination.

As a measure of the pigment concentration, the intensity of fluorescence was used instead of the absorption. One advantage of measuring the fluorescence instead of the absorption lies in the fact that a considerably smaller amount of pigment is needed and therefore much less material. The measurement can even be performed on the tissue itself without any extraction if the tissue is carefully infiltrated before the determination (see preceding section), although determinations made on glycerol suspensions of ground tissues give more reproducible values.

Preliminary experiments on the transformation of protochlorophyll by illuminating intact barley leaves, or glycerol suspensions of ground leaves, confirmed the observation that the pigment conversion is a second-order reaction and demonstrated that fluorescence measurements can be used to determine the course of the process.

All the experiments so far made with intermittent light have failed to give evidence for the existence of a chemical reaction. Two series of experiments were



made. In the first series the light flashes had a duration of 3 milliseconds, followed by dark periods of 13, 30, and 63 milliseconds respectively. The percentage transformation was measured after various times and compared with the values obtained when continuous illumination was used. No significant difference could be seen between the two methods of illumination. The experiments were performed both at room temperature and at 0° C.

In the other series, both the light and the dark period were changed: light from 5.8 to 1240 milliseconds, dark from 51.3 to 10,760 milliseconds. Even these experiments gave no different results from those obtained with continuous illumination.

The light intensity used in these experiments was rather low, at least far below the saturation point for photosynthesis. It is not known whether this light reaction also has a saturation intensity. The next step in these experiments will therefore be to measure the percentage of transformation after a light flash of high intensity as compared with the percentage of transformation after the same total amount of light energy has been administered as lower light intensity during a longer period of time.

#### DETERMINATION OF THE ACTION SPECTRUM FOR THE EFFECT OF LIGHT ON THE CONSISTENCY OF CYTOPLASM

HEMMING I. VIRGIN

An effect of light on the consistency of cytoplasm together with some experiments to determine the action spectrum for this reaction were described in Year Book No. 52 (p. 160). The effect of light is shown when the leaf is centrifuged. The particles normally suspended in the cytoplasm are thrown down in the exposed but not in the unexposed part of the leaf. These investigations have now been completed, and an action spectrum for the process has been obtained.

As in earlier experiments, leaves of *Elodea densa* were used, since this plant

material seems to give the most uniform results. The light effect has been established also in many other plants.

The experiments described in the previous Year Book resulted in an action spectrum very much like that for the phototropic phenomena, i.e., a spectrum similar to the absorption spectrum in visible light for  $\beta$ -carotene and riboflavin. Because of great variations in the plant material used, however, it was obvious that by the method described it was not possible to register such small fluctuations in the light response that peaks in the spectrum could be established if they do indeed exist. A new method was therefore worked out, with great hope that it would make it possible to register even small peaks in the spectrum.

From previous investigations it is known that the effect of light on the cells is strictly local; that only cells or parts of cells which have been illuminated show an effect after centrifugation. It should therefore be possible to get the action spectrum reproduced on the leaf itself by projecting a small spectrum on a homogeneous area of the leaf, then centrifuging the leaf. The spectrum used for this purpose should have equal energy at each wave length, and at a given wave length the light intensity should be very high at one side of the spectral strip and very low at the other side. Thus, the response of the differently illuminated plant cells should not depend on a fixed quantity of light energy received, but rather on a quantity of light that will be different at each wave length. Strong response would result in complete displacement of the cell contents after centrifugation. Weak response would give just a slight shift of the cell contents, or, since the all-or-none law seems to hold true here, the border between cells showing complete displacement and cells showing no change after centrifugation should follow a curved line representing the action spectrum.

An apparatus for producing such a spectrum was built according to the following

scheme. The light from an incandescent lamp given a small amount of overvoltage was focused on a slit having the shape of an isosceles triangle standing on its base. From the slit the light passed a system of lenses and a transmission grating. At the place for the image of the spectrum of the first order thus obtained, a wedge was inserted consisting of a photographic plate, gradually darkened, to compensate for the increase of energy toward the red part of the spectrum. The light then passed two further systems of lenses which decreased the image of the spectrum to a size  $1.25 \times 6$  mm. The spectrum thus obtained was made to fall on the leaf, which was placed behind aluminum foil with an opening a little narrower than the spectrum itself. In order to find the exact position of the different wave lengths of the spectrum projected in the opening of the aluminum foil, the incandescent lamp could be exchanged for a mercury lamp. The line of the mercury spectrum then showed up clearly in the opening, and by measuring the distance between the mercury lines 436 m $\mu$  and 546 m $\mu$  on a photographic plate inserted at this place, the exact position could be calculated. To get a reference point on the illuminated leaf, a notch was made in the aluminum frame at the place for the 436-m $\mu$  mercury line. A picture of this notch then appeared on the centrifuged leaf, and the position of the other wave lengths could be calculated. After many experiments to find out the best shape of the slit and the best combination of illumination time and intensity, centrifugation time and force, it was found that illumination for 4 minutes followed by centrifugation for 50 seconds in an angle centrifuge at a force of 1500 times gravity gave the best results. The slit had an angle of  $50^\circ$ , and the average light energy in the spectrum was 1095 ergs per cm<sup>2</sup> per sec.

After centrifugation the leaves were inserted in a projector giving an enlarged picture, which was photographed.

The individual cells of a leaf of *Elodea*

*densa* are large in comparison with the whole illuminated area. This implies that the pattern of the action spectrum after centrifugation obtained from just one picture is disturbed very much by the cellular pattern. This disadvantage could be diminished, however, by making a compound picture. Ten different single pictures were photographed on the same photographic plate, each picture receiving a tenth of the total exposure time. Owing to the afore-mentioned reference point, it was possible to orient the separate pictures in exactly the same way during the exposure.

The compound picture obtained in this way gave an action spectrum showing a very good resemblance to that obtained earlier. Contrary to what had been expected, however, no peaks in the spectrum could be found. On some single pictures it is possible to imagine two peaks, one at about 460 m $\mu$  and the other at about 490 m $\mu$ , but in the combination of many pictures this pattern is lost. It is, on the other hand, not possible to get a reliable idea of the spectrum from a single picture.

Thus, even if the original aim of the investigation was not fulfilled, i.e., to register small peaks in the action spectrum if there are any, the results of the experiments have nicely confirmed the individual response of single cells, showing that no transmission of stimulus takes place from an illuminated to an unilluminated area. The spectrum obtained in this way also shows a good agreement with that obtained by other methods.

#### CHARACTERISTICS OF A HIGH-TEMPERATURE STRAIN OF CHLORELLA<sup>1</sup>

CONSTANTINE SOROKIN AND JACK MYERS

Isolation of a number of high-temperature strains of *Chlorella* and characteristics of the growth of one of them (Tx 71105) as a function of temperature were reported previously (Year Book No. 51, 1951-1952,

<sup>1</sup> This work is being done at the University of Texas.



pp. 141-142). A continued study of some metabolic and cellular characteristics of one of them is the subject of the present report.

*Chlorella pyrenoidosa* and Tx 71105 were submitted to a comparative study of effects of temperature on photosynthesis, endogenous respiration, and glucose respiration. Each strain was grown in a continuous-culture chamber at its optimum temperature for growth (25 or 39° C). The time course of each of the above processes was observed by Warburg manometry at temperatures ranging from 15 to 50° C. The resultant families of curves show that temperature inhibition for each of the processes studied occurs at temperatures above the optimum temperature for growth. The temperature optima for photosynthesis, endogenous respiration, and glucose respiration, as observed in the first hours, occur at about 32° C for *Chlorella pyrenoidosa* as compared with its temperature optimum for growth of about 25° C. For Tx 71105 the temperature optima for the same three processes occur at 41° C as compared with 39° C for growth. Clearly the temperature limitations in both strains are determined, not by photosynthesis or respiration *per se*, but by other processes bound with growth.

The photosynthetic behavior of Tx 71105 is extremely sensitive to conditions of its previous history. The phenomenon is illustrated by the data given in table 4. Because of the complex geometry of the illumination, the values for light intensity have relative significance only. It will be noted that cells grown at higher temperature and lower light intensity have a lowered capacity for photosynthesis. Such cells also become light-saturated at rather low intensities. An explanation is found for the observation of Milner (Year Book No. 51, 1951-1952, p. 142) that Tx 71105 gives a yield of cells per day no greater than that of *Chlorella pyrenoidosa* when grown in dense cultures. At the low average light intensity experienced in dense cultures and in the temperature range 25

to 39° C, Tx 71105 has both a capacity for photosynthesis and a growth rate comparable with those of *Chlorella pyrenoidosa* at 25° C.

The maximum value of photosynthetic rate in the above data is the largest yet reported. The figure 180 mm<sup>3</sup> O<sub>2</sub> per mm<sup>3</sup> cells per hour can be expressed alternatively as follows: a cell of Tx 71105 can process in one hour an amount of carbon equivalent to one-third of its own dry weight or two-thirds of its own carbon content.

TABLE 4

INFLUENCE OF GROWTH CONDITIONS ON THE  
MAXIMUM PHOTOSYNTHETIC RATE

| PREVIOUS CONDITIONS OF GROWTH<br>IN CONTINUOUS CULTURE<br>APPARATUS |                             |                | LIGHT-SATURATED<br>RATE OF PHOTO-<br>SYNTHESIS                |
|---|-----------------------------|----------------|---|
| Temp.<br>(°C)   | Light inten-<br>sity (f.c.) | Growth<br>rate | (mm <sup>3</sup> O <sub>2</sub> /mm <sup>3</sup><br>cells/hr) |
| 25  | 60                          | 0.7.....       | 170   |
| 39  | 60                          | 0.6.....       | 50  |
| 39  | 240                         | 3.0.....       | 180   |

Further study was concerned with the phenomena of cyclic changes in the course of photosynthesis and respiration parallel to the cycle of life history of a cell. Such a cycle consists in increase in size of a small daughter cell to a large mother cell by assimilatory processes, and the subsequent division of the mother cell to a number of daughter cells. Metabolic changes accompanying the cycle have been demonstrated by Neeb (1952) in *Hydrodictyon*, and by Tamiya and coworkers (1953) in *Chlorella ellipsoidea*.

The steady-state population of the strain Tx 71105 in a continuous-culture apparatus was converted into a population made up almost entirely of small cells by a regimen of light and dark exposures ending in a dark period. Illumination of 240 foot-candles, known to be light-saturating for growth, was then applied. Samples of the suspension were removed at zero time and at various intervals for determination of

the time course of changes in endogenous and glucose respiration, photosynthesis, chlorophyll content, dry weight, and cell dimensions. When referred to a single cell, all these characteristics show a regular increase during the period of increase in size to large cells. When referred to cell volume or dry weight, the other characteristics follow a more complicated time course.

Small cells (autospores), prevented from growth by being kept in the dark, have rates of endogenous and glucose respiration of 2.5 and 11.1 mm<sup>3</sup> O<sub>2</sub> per mm<sup>3</sup> packed cells per hour. After the cells are induced to grow by transfer into light, the rate of respiration increases rapidly, and for endogenous respiration more than doubles after 10 minutes of illumination. After less than one hour of illumination the rates of both endogenous and glucose respiration reach their peaks (9.0 and 15.9 mm<sup>3</sup> O<sub>2</sub> per mm<sup>3</sup> cells per hour, respectively) and then undergo a slow and steady decline. In prolonged manometric runs the rate of endogenous respiration decreases with the time, the speed of the decline showing a dependence on the duration of previous illumination and presumably on the amount of stored photosynthate. Cells 8 hours old (observed after 8 hours of illumination) have enough stored material to support a high and constant rate (about 9 mm<sup>3</sup> O<sub>2</sub> per mm<sup>3</sup> cells per hour) of endogenous respiration for about 2 hours.

The rate of photosynthesis also undergoes considerable change with the age of the cells, but follows a time course different from that of the respiration rate. Small cells kept in the dark have a low light-saturated photosynthetic rate of about 90 mm<sup>3</sup> O<sub>2</sub> per mm<sup>3</sup> cells per hour. Cells 1 hour old, which already have passed their peak in respiration rate, increase their photosynthetic rate only to 108 mm<sup>3</sup> O<sub>2</sub> per mm<sup>3</sup> cells per hour. A maximum in the photosynthetic rate is reached in cells about 3 hours old. The increase in photosynthetic rate during the first 3 hours of

growth surpasses that in dry weight, chlorophyll content, and rate of respiration. After 3 hours of cell growth the photosynthetic rate decreases with time, falling to about 100 mm<sup>3</sup> O<sub>2</sub> per mm<sup>3</sup> cells per hour after 8 hours of illumination. If observations are carried beyond 8 hours, cell divisions begin and the population is no longer homogeneous.

As is seen from the above data, the cells most different in their photosynthetic capacity are cells from the dark and those 3 hours old. Light-intensity curves for these two types of cells display differences not only in maximum photosynthetic rates at light saturation (90 mm<sup>3</sup> O<sub>2</sub> per mm<sup>3</sup> cells per hour for cells from the dark versus 170 for cells 3 hours old), but also in the slopes of the light-limited region of the curves.

Description of the changes in metabolism throughout the life cycle is not yet complete. Because of its importance to an understanding of the basic features of algal growth, study of the cyclic metabolic events accompanying the life cycle is being continued.

#### ABSORPTION OF INORGANIC NUTRIENTS BY SCENEDESMUS<sup>2</sup>

ROBERT W. KRAUSS

In order to obtain reliable data concerning the efficiency of algae growing in mass cultures, a medium must be provided which will sustain optimum growth for a long time. This requires, first, the identification of all the nutrients essential to growth, and, second, a study of the rates at which each is absorbed and incorporated in the metabolic system of the cell. The approach to the latter problem was outlined in an earlier report (Year Book No. 52, pp. 167-169). The rates of absorption and resupply have been determined for the major nutrients nitrogen, phosphorus, sulfur, potassium, and magnesium in a typical unicellular green alga,

<sup>2</sup> This work is being done at the University of Maryland.



*Scenedesmus obliquus*. Data have also been collected for the micronutrients iron, calcium, manganese, zinc, copper, and cobalt. These metals can be added to media in relatively small concentrations, but must be maintained in a form available to the alga. This difficulty, of major importance in the nutrition of higher plants as well, is met through the use of chelating agents such as ethylenediamine tetraacetic acid (EDTA).

Two problems present themselves when chelating agents are used. It is necessary to determine the concentration of each element which will permit optimum growth for a limited period, and also to establish the replacement regimen required to maintain the maximum rate for an indefinite time. Experiments designed to resolve both questions have been conducted. The optimum concentrations of metals introduced as inner complex salts have been determined by means of the multiple culture apparatus (Year Book No. 51, 1951-1952, pp. 146-147). They are 9 ppm for Fe and Mn and 3 ppm for Ca, Zn, Cu, and Co. These levels insure maximum growth for a 10-day period. Media employing ethylenediamine dihydroxydiacetic acid have also been tested. The diol proves to be especially useful in preventing iron precipitation in alkaline solution.

The micronutrients are absorbed in a ratio quite different from that provided by the medium. Spectrographic analyses of cells grown in mass cultures supplied with chelated micronutrients were obtained with the co-operation of the American Spectrographic Laboratories. Analyses showed the cellular composition early in the culture period to be as follows: Fe, 0.079; Mn, 0.010; Ca, 0.051; Zn, 0.002; Cu, 0.004; Co, 0.00004 per cent dry weight. Later in the culture period, lower levels of Ca, Mn, and Cu were observed, indicating a progressive removal of these elements during periodic harvest.

A replacement formula has been calculated from these analyses and tested in mass cultures maintained under controlled

light and temperature. Yields of 80 to 90 per cent of the maximum were sustained by the formula, which returns to the cultures the amount of each nutrient removed in the daily harvest.

One major question remains unanswered. Are the metals removed from the chelate prior to absorption, or does the entire complex pass through the cell membrane? If the former were the case, a re-supply of metals alone would be necessary, but if the alternative holds, then both metal and chelating agent would be required. Our experiments have been conducted on the assumption that the entire complex is absorbed, and the metals re-supplied to the cultures have been in the chelated form. Experiments with higher plants support this view. The continued addition of a chelating agent may, however, reduce growth. It is now necessary to clarify this point by means of C<sup>14</sup>-labeled EDTA. Both the practical demands of nutrient replenishment and the theoretical considerations of chelate absorption and metabolism demand more detailed study of this question.

#### X-RAY-INDUCED MUTATION IN CHLAMYDOMONAS<sup>3</sup>

DONALD F. WETHERELL AND ROBERT W. KRAUSS

Obtaining an alga with superior characteristics for mass culture may be achieved either by screening species isolated from natural environments or by selection of artificially induced mutants. The latter approach may be especially valuable if the mutants are produced in an alga which, unlike *Chlorella*, is sexual and heterothallic, for it may then be possible by combining several desirable characteristics to develop an organism that will fit a given set of biochemical and cultural requirements. To obtain basic data concerning mutation in heterothallic algae, experiments were begun on the production and characterization of biochemical mutations

<sup>3</sup> This work is being done at the University of Maryland.

in *Chlamydomonas eugametos* and related species.

As a mutagenic agent X-irradiation is especially desirable because of the precision with which dosage may be controlled, and because of the available information concerning its effect on the genetic mechanism of higher plants. A new technique was required, however, to permit standardization of dosage and equivalent irradiation of single cells. To accomplish this, the test organism—previously on nutrient agar—is made motile by flooding with distilled water and by subsequent incubation in the dark for 3 hours. Dilute suspensions of the motile algae are deposited on a plastic, micropore membrane filter by vacuum filtration. The membrane, with approximately 1000 cells distributed on its surface in a single plane, is placed on agar or silica gel in a Petri plate. A thin layer of cellophane is temporarily substituted for the Petri plate cover before irradiation. Irradiation is carried out with a General Electric Maximar-100 X-ray tube. Run at 100 kvp and 5 ma, the output of unfiltered radiation is 920 r per minute at the working distance of 30 cm from the tube target. The inherent filtration of the tube window is equivalent to 0.1 mm of aluminum. After the period of irradiation, the membrane is transferred to any desired medium for incubation. The progeny of each irradiated cell may be observed during colony formation, and later are transferred to various media for complete characterization. Survival data plotted against dosage show that the percentage of cells killed rises linearly from zero at 1000 r to 90 at 7000 r. The curve is less steep beyond 7000 r, but less than 0.1 per cent survive at 11,000 r.

A conspicuous feature of irradiated *Chlamydomonas* is the large number of morphologically abnormal colonies. The relative number of abnormals is proportional to the dose, increasing from less than 1 per cent on control plates to 15 per cent at 5000 r and 35 per cent at 9000 r. These abnormal-appearing colonies have

been examined critically for biochemical mutation, for even on a complete medium an organism with altered metabolism should grow more slowly than its autotrophic counterpart. Study of isolates from the normal and abnormal colonies supports this hypothesis.

Testing for nutritional mutation is carried out by photometric comparison of growth in a minimal inorganic medium and in one containing amino acids, organic acids, nucleic acids, sugars, and vitamins. Isolates showing large differences in behavior on the two media are subcultured in inorganic media containing separately each of the five organic components. The isolate is finally screened in media containing the individual, pure chemicals of the class which has proved active.

Thus far, isolates showing differences in growth on complete and minimal media have come chiefly from the medium-sized abnormal colonies; the very small abnormal colonies have grown slowly in subsequent culture, regardless of the medium; the normal-appearing colonies have grown vigorously in all media. Typical mutant response is the attainment of logarithmic growth on complete medium within 2 days; parallel cultures on minimal medium show little or no visible growth for 6 to 10 days. An intriguing characteristic of some of the mutants is their capacity to grow rapidly in minimal medium once this long lag phase has been overcome. Subculture has established the fact that mutants which have become readapted to minimal medium show no such lag in subsequent development. Transfers from complete media, however, always show the long lag period in minimal media.

The remarkably large percentage of abnormal colonies and the correlation with dosage suggest that heritable damage other than gene or gene-simulating mutation has occurred. *Chlamydomonas* has a typical flagellate centriole-centrosome apparatus which is closely associated with mitosis. Damage to this or other structures important in cell division is a likely explanation



for some of the abnormal growth. This explanation seems especially cogent in consideration of a mottled type of colony frequently found in irradiated *Chlamydomonas Reinhardtii*. The mottled or checkerboard appearance is due to unequal distribution of cell material, especially chloroplasts, during division.

In view of the possibility that irradiation damage may involve heritable mitochondrial degeneration, centrosome destruction, or chromosomal disruption, as well as gene mutation, a straining technique has been adopted for examining mitotic figures, which should lead to an explanation of this phenomenon.

#### A DERIVATIVE SPECTROPHOTOMETER

C. S. FRENCH, ALLEN B. CHURCH, AND  
RICHARD W. EPPLEY

The absorption spectra of photosynthetic cells can be used to investigate changes in the photosynthetic pigments and in their associated cytochrome enzymes during the process of photosynthesis in living cells. This finding of Dr. Duysens has led to an attempt to develop a modified method for automatically recording small absorption peaks. It is believed that an extension of these studies to various fractions of disintegrated chloroplasts may be of value. A spectrophotometer which will plot the first derivative of transmission with respect to wave length is under construction. The direct recording of the derivative instead of the transmission curve should be a very sensitive means for detecting small absorption bands even in the presence of other strongly absorbing constituents. In addition to its suitability for the particular problem at hand, the device should have considerable value for a variety of spectrophotometric work.

In brief, the device is constructed according to the following principles: Light from a tungsten lamp is passed through a grating monochromator with a wide exit slit. An image of this exit slit is projected upon another slit, in the center of which is

a vibrating reed. This reed is driven at 120 cycles per second from one side of the slit to the other, alternately exposing one beam and then another. These two beams are a few millimicrons apart in wave length. The two beams then pass through the sample, which is at the entrance to a white sphere containing a photomultiplier tube. If the two beams are made equal in intensity by adjusting the relative height of the two sides of the slit with the sample removed, the photocell shows no AC output having the frequency of the vibrating reed. If the sample is now inserted, the amplitude of the signal is directly proportional to the difference in transmission for the two adjacent wave lengths, and its phase shows which of the two wave lengths is absorbed more strongly. If now the center wave length of the two beams is swept through the spectrum, while the spacing between them is kept constant, and appropriate corrections are introduced, the resulting plot will be the first derivative of the transmission curve of the sample. Thus, in passing over a single absorption band the record gives first a negative and then a positive response with zero deflection at the peak of the band.

The wave-length sensitivity of the photomultiplier, the emission spectrum of the lamp, and the transmission of the monochromator are measured together by taking part of the beam before it goes through the double slit and chopping this beam at a higher frequency. This reference beam is also introduced into the integrating sphere so that it is detected by the same photomultiplier tube. The AC output of the photomultiplier tube therefore consists of two components of different frequency. These are selectively amplified by tuned amplifiers and are both rectified for use on a DC Brown recorder. The recorder is arranged as a ratio-recording instrument, so that it plots the low-frequency signal passing through the sample as a percentage of the high-frequency reference beam which passes around the sample. In principle,

this correction takes care of the height of the over-all sensitivity curve of the apparatus, but does not consider its variation in slope, which will presumably have to be determined by means of a blank run, thus giving a zero line from which deflections are measured.

In order to keep the amplifiers operating at approximately the same level of signal throughout the spectrum in spite of large intensity variations, the light is attenuated by an optical wedge which is driven by a servo system under the control of the high-frequency beam. This operates to keep the reference voltage from the photocell at a constant level regardless of the wave length used. The use of this adjustable intensity greatly reduces the demands on the linearity requirements of the amplifiers.

In addition to plotting the first derivative of the transmission curve of the sample against wave length, if one half of the double slit is closed, the instrument should

be usable as a conventional recording spectrophotometer to plot per cent transmission against wave length. Provision is made for driving through the spectrum at any desired speed, and the linear wave-length drive is coupled to the chart paper by a pair of Selsyns.

Most of the parts of the original design have been constructed, and the over-all machine is in the early stages of testing. Doubtless many modifications in detail, and perhaps in principle, may be necessary before much experimental work can be done with it, although the results to date are encouraging.

To aid in the interpretation of the derivative curves produced by the machine, a series of hypothetical curves for various combinations of overlapping absorption bands are being prepared. Each combination is being plotted as extinction, transmission, and derivative of transmission by the curve analyzer.

## PALEOBOTANY

RALPH W. CHANEY

The use of modern vegetation as a basis for a better understanding of fossil floras has been discussed in previous reports, and has long provided the approach of our group to the interpretation of the Tertiary plant record in western North America. Such middle Tertiary floras as the Mascall and the Bridge Creek, from the John Day Basin of Oregon, contain so many tree and shrub species with living relatives as to indicate a remarkably close approximation to the modern forests of eastern North America and eastern Asia. Assuming that the environmental requirements of these ancient plants were essentially the same as those of groups of similar plants now living, we are able to reconstruct the climate and topography of the past with great accuracy. Wide forest studies in many parts of the northern hemisphere are furnishing a basis for many such comparisons, largely with temperate vegetation but also with some fossil units whose relation-

ships are to forests now living at lower latitudes. Particularly significant in a study of the development of vegetation in western North America during the past is information regarding modern forests on the borders between the tropical and temperate zones. In these areas of overlapping forest types, known as ecotones, lies the solution to many of the problems of Tertiary floras which contain puzzling mixtures of trees.

The ecotonal relationships of forests which live on the southern margin of the temperate zone in North America have been studied on numerous occasions during the past quarter century, and it has been possible to acquire a sound working knowledge of the latitudinal and altitudinal occurrence of many of the genera so situated in Mexico and Central America. Difficulties arise, however, when such studies are carried to northeastern Asia, a region of critical importance because many Tertiary plants once cosmopolitan are now



found living nowhere else. For lowland vegetation in the Orient has been largely removed to make way for agriculture, and forests are disappearing even from middle and high elevations. A beginning was made in the solution of this problem in 1948, when the broad-leafed evergreen forest of low altitudes in central China was observed ranging up into the temperate forest at about 3000 feet, where *Meta-sequoia* and its deciduous associates are dominants. Because of political conditions it has been impossible to return to this critical area for a more detailed study, but field work has been carried on subsequently in Japan, where the forests show a similar resemblance to Tertiary vegetation.

During the summer of 1953 a visit was made to Izu Peninsula, on the east coast of central Honshu, with staff members of the Japanese Forest Service. This is a mountainous region, with some primary forests at upper levels, and at least one lowland tract of native trees in the Nekko Forest Preserve; a score or more of the common angiosperm genera of the Oregon Eocene were observed in this Preserve, but there are no higher elevations where transition from this subtropical forest into a temperate forest above might be observed. It was possible, however, to find the upland assemblage unusually well represented on the slopes below Amagi Pass, a few miles to the south, at altitudes from 1800 to 2500 feet, and to note its relations to a lowland forest which here has been largely cut off, but which is essentially the same as that in the Nekko Forest Preserve. Twenty or more of the common temperate genera of the middle Tertiary were observed at higher levels, including alder (*Alnus*), hornbeam (*Carpinus*), beech (*Fagus*), katsura (*Cercidiphyllum*), and maple (*Acer*). At lower levels in this forest, there is some penetration of evergreen oaks (*Quercus acuta*), laurels (*Lindera*, *Neolitsea*), and other warm-temperate to subtropical trees and shrubs, but for the most part they are confined to altitudes below 1300 feet. This modern distribution gives

an illuminating picture of early Tertiary forest zonation in western North America, where a few leaves of temperate trees are often found associated in the fossil record with the dominant subtropical foliage characteristic of the Eocene lowland forests, or where a minority representation of tropical species may occur in younger forests predominantly temperate. At best only fragments of the past can be found in the fossil record, and the vegetation of today always shows changes where time has left its mark. These segments of the Tertiary surviving in Japan, however, assume high significance in the proper interpretation of ancient forests, for they more nearly resemble tropical-temperate ecotones of the past than do any others which are available for study.

Problems involving the distribution of Tertiary conifers and their living allies have led to a survey of the pattern of *Sequoia* occurrence in later geologic time. Now restricted to a narrow belt along the Pacific coast where freezing temperatures are infrequent, the coast redwood (*Sequoia sempervirens*) shows evidence of a former wider distribution to the east; small outlying groups of trees may be found in the inner Coast Ranges of central California, the easternmost of which falls in the drainage of the Sacramento River. Equally suggestive is the discovery of fossil remains of its immediate ancestors at progressively older stratigraphic levels as far east as North Dakota.

Unlike the coast redwoods of today, which have their best development near sea level, the ancestral *Sequoia affinis* of the Tertiary period appears to have lived largely in the uplands. In his recently published monograph on the Florissant flora of central Colorado (Carnegie Publication 599), MacGinitie has described the presence during the Oligocene of large trees of this species at an elevation of about 3000 feet, and has noted that their cones are much smaller than those of the living species. Current studies of the Blue Moun-

tains flora from the Miocene of eastern Oregon indicate a similar occurrence of *S. affinis* at altitudes well above sea level, and the cones here are also conspicuously small. In an effort to determine whether there is a correlation between cone size and habitat, several large samples of the cones of *S. sempervirens* have been collected and measured. Those which come from interior trees at relatively high altitudes are markedly smaller in length and diameter. By contrast, the cones of the oldest redwood, *S. dakotensis*, from the Cretaceous of North Dakota and Montana average much larger than those of the living species. Since no remains of *Sequoia* are known from shore deposits near the Pacific until middle Tertiary time, the suggestion may be made that this genus had its origin on the borders of the interior Cretaceous sea, and that uplift and resulting changes in climate have caused it to shift its range westward during the past hundred million years. It seems to have lived only at higher altitudes

in what is now the Rocky Mountains and the Columbia Plateau, and its small cones and certain foliage characters suggest that the climate there was not suited to its best growth. Ultimately reaching the ocean borders, it has found a favorable place for survival in spite of reductions in precipitation and lowered temperatures during later geologic time; for summer fogs and mild winter temperature provide the Cretaceous type of climate in which its earliest ancestor appears to have become first established. Several other living conifers with Tertiary equivalents may have followed a somewhat similar path westward, and are now numerous on the borders of the Pacific Ocean. Such longitudinal shifts in distribution are less readily recognized than the latitudinal shifts which have been extensively described in preceding reports, but are no less significant as indicators of past climates and mountain building, and in explanation of modern forest distribution.

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## DEPARTMENT OF EMBRYOLOGY

*Baltimore, Maryland*

GEORGE W. CORNER, *Director*

The past year has seen no changes in the permanent staff of the Department of Embryology, and no abatement of the program of research and publication.

In September 1953 the Director returned from Oxford, where he held the George Eastman Visiting Professorship for 1952-1953.

Dr. A. St. G. Huggett, Professor of Physiology at St. Mary's Hospital Medical School, London, arrived in September for a stay of nine months. He was the holder of a Fulbright travel grant, tenable as visiting Lecturer in Obstetrics at Johns Hopkins Medical School, and his work was aided also by a grant from the Baltimore Rh Laboratory. In the Department of Embryology Professor Huggett conducted, in close association with Dr. S. R. M. Reynolds, a study of placental function in the rhesus monkey. For this purpose the entire resources of the departmental monkey colony were put at his disposal, and a substantial grant for apparatus and materials was made by the Carnegie Institution of Washington. The important results achieved are summarized below, under "Research in Progress." Opportunity to study certain aspects of the same problem in human subjects was afforded by the Department of Obstetrics of the Johns Hopkins Hospital.

Dr. Catherine Neill of London worked at the laboratory as a Fellow of the American Heart Association.

Dr. Brenda M. Schofield was appointed to a fellowship of the Carnegie Institution of Washington, beginning in January 1954 at the conclusion of her first year's stay on a Fulbright fellowship, and continuing until June 1954, when she returned to England.

Dr. Vittorio Danesino of the University

of Naples was appointed to a fellowship of the Carnegie Institution October 1, 1953, at the expiration of his Fulbright fellowship; he returned to Italy in June 1954.

Dr. Efstathios J. Kokorikos of the University of Athens, who was also appointed to a fellowship of the Carnegie Institution October 1, 1953, concluded his work and returned to Greece in June 1954.

Dr. Beni Horvath, who joined the Department as a Fellow of the Carnegie Institution of Washington July 1, 1952, resigned in September 1953 to accept a permanent position at the National Institutes of Health, Bethesda, Maryland.

Dr. W. M. Paul came in January 1954 from the University of Toronto to work with Dr. S. R. M. Reynolds on the physiology of the uterus. He is the holder of a fellowship awarded by the R. Samuel McLaughlin Foundation.

The work done by all these temporary members of the Departmental group will be summarized in the sections on "Research in Progress" and "Published Research" in the following pages.

Dr. Chester H. Heuser, Research Associate, returned to the laboratory for two months in the summer of 1953, continuing his work on early human embryos.

Dr. E. Carl Sensenig of the University of Alabama spent several weeks in the laboratory in the summer of 1953 following up his previous studies on the development of the skull and spinal column in human embryos.

Dr. Perry W. Gilbert of Cornell University again made two visits of a few days each to consult about the illustrations of his forthcoming monograph on the development of the external muscles of the human eye.

The following persons also made visits



of several days to a fortnight, making use of the collections of embryos and other facilities, for the purposes indicated:

Dr. E. C. Amoroso, Professor of Physiology at the Royal Veterinary College, London, June 1954: to give a talk and to consult about placental morphology and physiology.

Dr. Geoffrey S. Dawes, Director of the Nuffield Institute for Medical Research, Oxford, England, August 1953: to observe the program of research.

Dr. R. Caldeyro-Barcia, University of Montevideo, June 1953: to consult and

collaborate with Dr. Reynolds on problems of uterine physiology.

Dr. A. Tortora, Naples, Italy, September 1953: to observe the program of research.

#### CONTRIBUTIONS TO EMBRYOLOGY, VOLUME 35

At the time of this report, volume 35 of the Contributions to Embryology is in page proof, and will be published in 1954. The table of contents was printed in Year Book No. 52, page 185.

The assembling of materials for volume 36 will begin in the summer of 1954.

### RESEARCH IN PROGRESS

#### EMBRYO, FETUS, UTERUS

*Early human embryos.* Dr. Chester H. Heuser, Research Associate, continued his accumulation of data, looking toward the preparation of a companion volume to Streeter's *Developmental Horizons in Human Embryos*. Dr. Streeter began this descriptive classification of human embryos at horizon xi, leaving earlier stages for consideration later. Before his death in 1948 he published all but horizons xix to xxiii. These were completed from Dr. Streeter's notes by Dr. C. H. Heuser and Dr. Corner, and subsequently horizons xi to xxiii were gathered into a single volume (Carnegie Institution of Washington, Embryology Reprint Volume II).

The draft of horizon x is now being prepared by Dr. Heuser, who is also assembling the materials for the earlier horizons.

*Development of the pulmonary veins.* Recent advances in cardiac surgery have made possible the repair of congenital defects in the septum between the atria of the heart. In patients with such defects, the pulmonary veins sometimes drain anomalously into the right side of the heart. Consideration of the embryology of such cases raises the question whether the common pulmonary vein normally enters the sinus venosus in the early embryonic stages and then is shifted to the

left atrium following the development of the atrial septum, or is from the first connected directly with the left atrium. Dr. Catherine Neill has studied many embryos in the Carnegie Collection and has made plastic-sheet reconstructions of three hearts from embryos of the 4th to the 6th week. These show that the common pulmonary vein develops as an outgrowth from the medial superior wall of the left atrium which unites with the plexus of vessels developing in the lung bud. No evidence of a primary connection with the sinus venosus was found. The results of this investigation are being prepared for publication in a pediatric journal.

Dr. Neill has also made a contribution to the physiology of the embryonic heart by recording electrocardiograms of rabbit embryos, correlating this electrical evidence of beginning function with the first appearance of the heartbeat and with the anatomy of the embryonic heart, i.e. the development of specialized cardiac conducting tissue and nerve fibers. Such studies have been made previously on embryonic chicks, but not apparently on young mammalian embryos. Before 9 days Dr. Neill obtained no electrocardiographic evidence of the heartbeat. At 10 days, when the heart is an S-shaped loop, and the circulation of blood is beginning, simple polyphasic records with recogniz-

able P waves were obtained. Nerve fibers and conducting tissue are not clearly discernible until 16 days, a fact which indicates that the intrinsic biochemical reactions of the developing heart muscle are fundamental in the onset of pulsation and of electrical activity.

Colored motion pictures of living 9- and 12-day rabbit embryos were made for Dr. Neill, recording the heartbeat before and after blood is present.

*Ductus arteriosus.* Guided by the conclusions drawn from an earlier study of the arrangement of tissue elements in distended and constricted umbilical arteries and veins, made by Dr. Anna W. Chacko and Dr. Reynolds (summarized in Year Book No. 52), Dr. Danesino has correlated the histological structure of the walls of the ductus arteriosus with the age of the fetus (5 months to term) and with the degree of constriction of the ductus. Certain parts of the vessel are found to constrict more than others. Serial sections of the ductus arteriosus, obtained from 60 fetuses and newborn infants, were made available for this study by Dr. I. H. Rehman of the University of Southern California.

The disposition of elastic tissue and the pattern of the smooth muscle bundles indicate that the greater (superior) curvature of the ductus contributes more to the constriction and shortening which effect the closure of the ductus after birth than does the lesser (inferior) curvature. Particular significance is attached to the presence of a mucoid substance in the walls of the ductus arteriosus comparable to Wharton's jelly in the umbilical cord.

*Sex differentiation.* Dr. R. K. Burns was in Florida for about 8 weeks in February and March, collecting opossums and conducting experiments on the modification of the developing gonads by very early administration of the female sex hormone estradiol to newborn young. Serial sections of the reproductive tracts of the experimental animals, which are being prepared at the time of this report, indicate that definitely positive results have been ob-

tained. Modification of the embryonic testes has in some of the injected animals gone so far as to make them resemble ovaries not only in general structure, but also in the presence of primordial follicles containing ovocytes.

Dr. Burns also collected and prepared a series of young opossums for study of the rete canal system of the gonads. He resumed a previously begun study of the role of the genetic sex constitution of the individual in regulating the rate of growth of the sex primordia under hormonal stimulation. In collaboration with Dr. Alfred Jost of Paris, a study was begun of the effects of sex hormones on the functional activity of the pituitary gland of the opossum.

During his stay in Florida, Dr. Burns, aided by Mrs. Burns, obtained a great deal of information on the breeding season, size and frequency of litters, age of first pregnancy, and population density of breeding opossums in the region of Welaka, Putnam County. This work was carried out at the Conservation Reserve of the University of Florida, by courtesy of the Department of Biology of that institution.

*Mechanisms of implantation.* Dr. Bent G. Böving has made observations on the spacing of the implanted embryos of the rabbit in the long, tubular uterine horns. Statistical analysis indicates that natural spacing is so directed that the embryos are implanted at approximately equal distances from one another and from the ends of the horn, the distances varying with the number of blastocysts. Glass beads introduced into the uterus are moved and spaced more or less as are the blastocysts, if they are of appropriate size, i.e. between 1 and 4 mm in diameter. The spacing evidently depends on a physical stimulus by each individual blastocyst and an intrinsic mechanism of the uterus.

Dr. Böving also devoted much time during the year to further study of the remarkable finding, described in last year's annual report (Year Book No. 52), of a localized alkaline reaction in the wall of the rabbit



blastocyst opposite to the embryonic rudiment. He has determined the chemical nature of the alkaline reaction by studying crystalline deposits which form in the region of the spot when the blastocyst is removed to a glass slide and allowed to dry. By chemical methods and X-ray crystallography (kindly done by Professor J. D. H. Donnay of the Johns Hopkins University), these crystals are shown to be calcium carbonate. There is much sodium chloride and potassium chloride in addition, but the calcium carbonate crystals may readily be distinguished under polarized light. The calcium carbonate is believed to combine with and neutralize acid products of blastocyst metabolism during the several days which elapse before there is effective gas exchange between the embryo and the uterus. When the accumulated products of metabolism can pass into the maternal circulation, an alkaline reaction results at the site of transfer.

Such sites of transfer have been visualized by the injection of silver nitrate solution into the maternal circulatory system. Unusually heavy precipitates occur in those epithelial cells which line the central lumen of the uterus near a blastocyst and have a blood vessel at their base. It is precisely to such epithelial cells that the blastocyst adheres when it attaches to the uterus, and there is reason to believe that the adhesiveness results from the action of alkalinity upon the noncellular membrane which surrounds the blastocyst. This membrane had previously been identified with the zona pellucida, but considerations of volume and chemical behavior showed that it is derived, not from the original egg covering of that name, but from the well known "albumen layer" secreted around the ovum by the tube, plus an additional covering contributed by the uterus. This outer covering has been called by Dr. Böving *gloiolemma* ("adhesive layer").

The sites of transfer revealed by silver also correspond in location and dimension to the sites of subsequent trophoblastic invasion of uterine epithelium. The prox-

imity of trophoblastic invasions to vessels is therefore attributed by Dr. Böving to adhesiveness induced near vessels by the release there, by the trophoblast, of an alkaline reaction. Certain chemical and mechanical aspects of the invasion itself and possible relations to invasion by malignant tissue are also suggested by these studies, and it is planned to continue work in this direction.

*Pathology of the embryo and fetus.* Dr. Elizabeth M. Ramsey examined during the year 98 specimens sent by 28 institutions and private physicians. Of these, 35 had sufficient value for research or teaching to justify preservation. Reports on all cases were made to the senders, including replies to the accompanying requests (which are increasing in number) for advice regarding the cause of the abortion, the expectancy of repetition, and means of preventing recurrence. Dr. Bartelmez continued to contribute advice and assistance in this work, which is done in the intervals of the busy research program of the laboratory as a public service and as a means of cultivating the interest of the medical profession.

The Sloane Maternity Hospital Study No. 2, on gestation, birth, and the first year of life, at the Columbia University-Presbyterian Hospital Medical Center, New York City, continues to use the services of Dr. Ramsey as a member of its Advisory Committee. Dr. Ramsey examined and reported on 19 fetuses originating in the study during the year.

#### STRUCTURE OF THE UTERUS AND OF THE PLACENTA

Dr. George W. Corner investigated the arrangement of the muscle layers of the rabbit's uterus, having in mind the relation of the structure of the uterus in its relatively gross aspects to the behavior of the organ as seen in the studies of Dr. Böving, Dr. Csapo, and Dr. Schofield, summarized in this report. By examination of sections and by dissection under the microscope, a pattern was revealed which is more com-

plicated than appears in the literature. Dr. Corner presented his findings as part of an informal conference given at the Ciba Foundation, London, during a brief visit to England in June 1954.

*Blood vessels of the endometrium.* Dr. G. W. Bartelmez devoted most of his time to the continuation of his careful examination of the blood vessels of the lining of the uterus of the rhesus monkey. A monograph on this subject is now in preparation. He finds evidence that the difference in the responses of the several zones of the endometrium to the ovarian hormones can be accounted for, in part at least, by differences in the pattern of the blood vessels in the respective zones. Counts made on 4 uteri show that the endometrium is supplied by 2000 to 3000 arteries. Three distinct beds of capillaries are fed by these arteries, viz., in the superficial layer, in zones II and III, and in zone IV. The superficial network, which becomes sub-epithelial during the progravid phase, is fed by the terminal branches of the coiled arteries. It has a relatively low threshold for estrogens, so that a superficial hyperemia is developed when the concentration of estrogen is high. It is drained by numerous venules, some of which pass directly to the deep venous sinuses (radial venules). Between it and zone IV (i.e., in part of zone II and zone III) the capillaries are in general longer than elsewhere in the endometrium, some of them reaching a length of almost 200 microns. Each coiled artery here has its own capillary bed and venules, with no such extensive anastomoses as in the superficial bed. The circulation in both these capillary beds is made intermittent by rhythmic constrictions of the coiled arteries. Zone IV is characterized by short capillaries fed by numerous small basal arterioles. There are at least 9 times as many of these as there are vessels which reach zone II.

Dr. Bartelmez reports that no evidence has been found of the existence of arterio-venous anastomoses, such as are described

in the human endometrium by some recent investigators.

The superficial resemblance of the low endometrium of late menstrual stages to the basal zone (IV) of other phases has led to the assumption, believed by Dr. Bartelmez to be incorrect, that all but the basal zone is shed during the menstrual phase. No evidence has ever been produced to show that there is extensive post-menstrual regeneration, which would be expected if there had been a heavy loss of tissue. This hypothesis, moreover, disregards the extensive involution which occurs just before and during menstruation. The loss of fluid, both blood and tissue juices, and the reduction in cell size are more important than tissue loss. Post-menstrually there is rehydration and reorganization of the zones injured by the menstrual ischemia. Detailed changes in the arteries, which Dr. Bartelmez is recording for publication, illustrate and corroborate this interpretation of the events of the menstrual cycle in the endometrium.

*Placenta.* Dr. Elizabeth M. Ramsey has continued her study of the blood vessels of the human placenta, previously mentioned in these reports. A preliminary statement of some of her results was presented at the annual meeting (April 1954) of the American Association of Anatomists and at the Cold Spring Harbor Symposium on Quantitative Biology (June 1954). In brief, neither of the chief current interpretations of the maternal placental circulation (Bumm, 1892; Spanner, 1935) is confirmed by the present analysis. Thus, taking issue with Bumm, Dr. Ramsey finds that arterial entries do not characteristically occur high on the maternal septa, but in the basal plate; and, contrary to Spanner's contention, she finds that basal veins are demonstrable all over the placenta, at all stages of pregnancy, and drainage is therefore by no means exclusively marginal. Applying physiological concepts to her own picture of the anatomical arrangements, Dr. Ramsey finds that both arterial inflow and venous drainage occur at the



base of the maternal placenta, the afferent and efferent streams being separated and the blood flow distributed evenly through the placenta by pressure differentials assisted by various features of the morphological structure.

To previous acknowledgments of generous contributions of human placentas in situ, by physicians and hospitals of Baltimore, special thanks should now be added for the contribution of 6 well seriated placentas by Dr. Irwin S. Kaiser of the University of Minnesota, Department of Obstetrics and Gynecology, a former Fellow of the Carnegie Institution of Washington.

#### PHYSIOLOGY OF THE PLACENTA

*Placental transmission of glucose and fructose.* A great deal of work has been done in ungulates, especially sheep, on the transmission and metabolism of glucose and fructose in the placenta. One of the leaders in this field is Professor A. St. G. Huggett of the University of London. His work has shown that glucose injected into a pregnant ewe appears at once in the fetal blood, and that there is also, after a time, an increase in the amount of fructose in the fetal blood. When fructose is injected into the mother, it appears at once in the fetus; and both these sugars, when injected into a fetus, cross the placental barrier from fetus to mother.

Whether similar processes occur in the human and other primate species has not been known. To study the question, Dr. Reynolds organized a group to work with Professor Huggett during the latter's visit. The team included two Fellows of the Department, Dr. Danesino and Dr. Paul; Dr. William L. Hartman, Department of Obstetrics, Johns Hopkins Hospital; and Dr. Francis P. Chinard, Johns Hopkins Medical School. The transfer of carbohydrates across the placenta was studied in monkeys, and also in women as opportunities occurred in the obstetrical clinic of Johns Hopkins Hospital.

In women, glucose is found to cross

promptly from mother to fetus, but no indication of a rise in fetal fructose has yet been observed. This point is being further explored. The placenta transmits fructose quite as readily as glucose.

In monkeys, on which experiments can be conducted and much more elaborate observations made, the situation appears to be like that in women. For example, when glucose is passing across the placenta from mother to fetus, additional radioactive glucose or fructose injected into the fetus appears within a few seconds in the maternal blood, having moved against the concentration gradient, although at a substantially diminished rate. Conversely, radioactive glucose or fructose can move against the gradient established by injection of  $C^{12}$  glucose or fructose into a fetus, again at altered rates of exchange. It appears that the primate placenta does not produce significant amounts of fructose, nor convert glucose into fructose in substantial amounts, nor transmit glucose or fructose preferentially, either as to direction or as to gradient of concentration. The fuller implications of such findings will be stressed in the published reports.

In connection with the foregoing investigation, Dr. Huggett, Dr. Paul, and Dr. Danesino studied the rate of glycogen formation in pregnancy, in the rabbit, by injecting radioactive glucose in order to determine whether it is later found in the placenta in the form of glycogen. Dr. Danesino followed up these experiments with histochemical studies on the distribution of glycogen and of phosphatase in the rabbit's placenta.

*Experimentation on the fetus in utero.* The foregoing work necessitated experimentation on fetal monkeys. These, however, are not accessible for experiment by the methods usually applied to other laboratory animals, for when the uterus is opened the placenta promptly separates, terminating the experiment. To circumvent this difficulty, a new method has been developed by Dr. Reynolds and Dr. Paul which opens up the way to physiological

study of the fetus in utero, with its amnion intact and with minimal trauma to the uterus.

The method rests on the fact that the rhesus monkey, like a number of other primates, usually has two separate placentas, the primary and secondary lobes being connected by large fetal blood vessels which lie on the inner surface of the chorion, outside the amnion. Exposing the uterus of the pregnant monkey under anesthesia, the investigators have been able, by ingenious methods of illumination and operative technique, to locate and deliver a segment of one of these large fetal vessels through a small incision in the uterine wall, without opening the amnion. By catheterizing the vessel with a fine plastic tube, which can be passed almost to the base of the umbilical cord, fetal blood can be obtained and fetal blood pressure recorded without serious interference with the placental circulation, the primary placenta having been left undisturbed. It has been possible to observe the fluctuations in the fetal heart rate and blood pressure produced by varying the intrauterine pressure, to withdraw repeated samples of fetal blood for analysis, and to inject substances directly into the fetus, without the intermediation of a placental barrier.

#### BIOCHEMISTRY OF PHYSIOLOGY OF UTERINE MUSCLE

During the year, Dr. Arpad Csapo's studies of the biochemistry and physiology of uterine muscle included a detailed investigation, with the co-operation of Dr. M. C. Goodall of the University of Michigan, of the relations between length and tension of muscle strips, between strength and duration of stimulation and the tension developed by resulting contraction, and between the load imposed on the contracting muscle and the velocity of shortening. The results of the experiments show that the main principles of uterine muscle contraction are similar to those operative in skeletal muscle, even though the kinetics is different in the slow-acting uterine

muscle. This finding makes possible increased exchange of ideas, techniques, and criticisms between workers whose experience has been gained from one or the other kind of muscular tissue respectively, and should increase the rate of discovery in both parts of the field.

Dr. Csapo continued his investigation of the role of inorganic ions in uterine muscle contraction and particularly in the control of such muscle by the ovarian hormones. By modifying the concentrations of potassium and sodium ions in the fluid surrounding the muscle samples, he finds that under physiological conditions the gradients of potassium and sodium concentration determine the function of the muscle by their effect on the activation of the contractile substance. The previously reported demonstration that progesterone affects the behavior of uterine muscle (e.g. the "staircase phenomenon") thus acquires a generalized explanation (see also the summary, under "Published Research," below, of Dr. Beni Horvath's study of potassium and sodium concentration in uterine muscle).

Dr. Csapo has carried out a very extensive series of experiments on pregnant rabbits, intended to elucidate the cause of the onset of labor. Labor was induced by injections of pituitrin at stated times in the last days of gestation. Progesterone, injected in suitable doses, was found to inhibit the onset of pituitrin-induced labor; by varying the interval between the injection of pituitrin and the time at which this hormonal support was afforded to the uterus, the latency period of progesterone action in the living rabbit was ascertained. On the basis of these experiments a working hypothesis of the control of myometrial function has been worked out, and will be expounded by Dr. Csapo at the International Congress of Obstetrics and Gynecology at Geneva, July 1954, and elsewhere. All of the year's work has been prepared for publication in various books, journals, and congress reports.

*Physiology of the uterus in vivo.* Dr.



Brenda M. Schofield studied the influence of the ovarian hormones on the isometric tension developed by the uterus in the living animal. A method was evolved whereby the uterine muscle could be directly stimulated electrically and its isometric activity recorded in situ. Dr. Böving gave valuable assistance in the construction of apparatus for this work. Experiments were carried out in parallel with Dr. Csapo's studies on the isolated muscle, and close qualitative similarity was demonstrated between the results obtained in vitro and in vivo. The behavior of the muscle and its response to electrical stimulation varies according to whether the hormone dominating the uterus is estrogen or progesterone. This was shown in rabbits which were castrated and treated subsequently with controlled amounts of the two hormones, and also in rabbits in early pregnancy, when the uterus is under progesterone domination.

This finding, that the action of the ovarian hormones on uterine muscle is essentially similar whether it is observed in isolated fragments of muscle or in the living rabbit, is important because of its bearing on the philosophy of investigation in this field. It is obviously necessary to isolate the various elements of a complex phenomenon (such for example as muscle contraction) in order to get at its fundamental elements. In these experiments by Dr. Schofield the process of resynthesis is seen, in which elementary processes observed in the isolated tissues are watched for, observed, and to a certain extent understood as they occur at a functional level in the complete organ.

Dr. Schofield also studied a problem of importance in connection with theories of the onset of labor, and ultimately in connection with the prevention of spontaneous abortion. This concerns the time interval between a single injection of progesterone and the consequent change-over of the uterus from the estrogen-dominated to the progesterone-dominated condition. Serious experimental difficulties arising from the

low solubility of progesterone in fluids which can be injected into animals were overcome by various expedients. The final conclusion is that the time required is in the neighborhood of 24 hours. In the living animal there is nothing analogous to the immediate fall in uterine tension which occurs in the isolated uterus after addition of progesterone to the bath.

*Physiology of the human cervix uteri.* Dr. Paul, with Dr. Reynolds, has embarked on a study of the contractility and distensibility of the nonpregnant human cervix uteri, and of pain produced by distention of the cervix. This is being done in collaboration with the Department of Gynecology, Johns Hopkins Hospital. The method depends on a new development, namely, the use of a specially designed long, narrow, internally supported balloon which is inserted into the cervical canal. This balloon is then distended, measuring by strain-gauge transducer the pressure required for given volumes of distention. The variability of cervical tone and contractility is now being studied, with a view to evaluating the action of drugs which are used to affect the autonomic nervous system for the relief of painful menstruation.

#### PHYSIOLOGY OF THE MALE REPRODUCTIVE TRACT, AND OF THE SEMINAL FLUID AND ITS ENVIRONMENT

Under Dr. David W. Bishop's leadership, work has progressed along several lines in the study of sperm environment and sperm reactions. Investigations have been conducted in collaboration with Dr. V. Danesino and Dr. E. J. Kokorikos, Fellows of the Carnegie Institution of Washington, with Dr. Seymour Katsh of the California Institute of Technology, and with Dr. Georgiana Seegar-Jones of the Johns Hopkins Medical School, all of whom plan to follow up certain aspects of the work in their respective laboratories.

Biochemical and chromatographic methods are being used to identify possible energy substrates in rabbit prostate and

seminal vesicle secretions. In order to obtain pure secretory fluid in moderate quantity, the technique of subcutaneous transplantation has been exploited in the mouse and in the rat. The findings indicate that carbohydrate is secreted mainly as free fructose, and to a lesser extent as free glucose, little or none being produced in the phosphorylated form, as was recently suggested by Scott *et al.* for the accessory-gland secretions of man and the dog. Thus no enzymatic conversion of these seminal components, in the rabbit at least, is required for their adequacy as metabolic substrates for spermatozoa at the time of ejaculation. This conclusion is further borne out by the demonstration of Dr. Kokorikos that neither the seminal vesicles nor the prostate gland (or their secretions) of the rabbit have more than a very low concentration of either acid or alkaline phosphatase.

The accessory-gland transplantation procedure has also permitted the direct determination of extratesticular sources of androgen. Adrenal tissue from young rats, transplanted into either male or female adrenalectomized castrates, secretes sufficient quantities of steroid to maintain for 30 days the secretory activity of adjacent seminal vesicle implants, but not of the host seminal vesicles. Adrenals in situ sustain accessory-gland transplants in either male or female castrates, and preliminary evidence indicates that the normal female rat's adrenal has a greater effect than does the male adrenal.

The transplanted ovary also produces sufficient androgen in young adrenalectomized castrates to stimulate male accessory-gland transplants, but the andromimetic effect is less than that of adrenal tissue and, as would be expected, far less than that of the testicular transplants.

The transplantation of these tissues to subcutaneous sites in the mammary area proved highly successful in the mouse and the rat, but failed in numerous attempts on the rabbit, guinea pig, and monkey. When adequate histological material is

available to confirm the preliminary results, the experiments will form the basis of a report by Dr. Bishop, Dr. Danesino, and Dr. Katsh. The latter plans to follow up two significant aspects of the above findings, the local "field effect" of hormonal activity and the possible alternative of chemical conversion of steroids, on the one hand, and direct androgen output by adrenal tissue, on the other. Dr. Danesino, who has been of inestimable help in the operations and in the detailed planning of experiments, is interested in studying further the development of ovarian transplants. These grafts show excellent growth and follicle development in both male and female castrated hosts. He proposes to investigate preovulatory changes and other ovarian modifications in such grafts.

The extraordinarily high concentration of the phosphatase enzymes in the prostatic fluid of some species, the significance of which however is not clear, encouraged the joint study with Dr. Kokorikos of the acid and alkaline phosphatases of the entire male and female genital tracts of the rabbit. Acid phosphatase, quantitatively determined by colorimetric methods, is most highly concentrated in the active testis, falls off a little in the upper epididymis, is somewhat less in the lower epididymis, and appears only in low concentrations in the vas deferens and prostatic tissue. The seminal vesicles and sperm either from the vas or in the ejaculate have inappreciable quantities of the enzyme. In the female tract the acid phosphatase appears only in cervical mucus of animals which have been treated with estrogen. Acid phosphatase thus appears to play a minor role in sperm function, but possibly a significant role in the formation and "maturation" of spermatozoa. Dr. Kokorikos plans to continue this study, to determine the extent to which the gradient in the male tract is due to the genital tissues and to the gametes respectively.

Alkaline phosphatase proved to be present in very high concentration in the upper



epididymis, a region of the male tract which is known to be metabolically very active. The lower epididymal tubules and the testis have less alkaline phosphatase activity than the rest of the male tract. The spermatozoa and the entire female tract, including cervical mucus, were extremely low in activity as measured by this technique. The determinations were carried out at pH 9.5 for alkaline phosphatase and pH 5.0 for acid phosphatase, using as substrates p-glycerophosphate and phenyl di-phosphopyridine. Pending the procurement of supporting histological data, based on cytochemical techniques, the results of this biochemical approach are being prepared for publication.

By means of a two-stage operative procedure in which the uteri and tubes of female rabbits are serially ligated one or two days before killing, adequate quantities of fluid may be collected for biochemical analysis. Hydrogen-ion concentrations have been determined on the secretions from all levels of the tract under various types of endocrine domination. Though the pH tends to remain constant at about 7.5 throughout the tract, one significant finding has been that estrogen depresses the pH of the upper tubal secretion about 0.3 of a pH degree. Sugar determinations on the tubal fluid indicate absence of fructose and only variable quantities of glucose in the female genital tract, a fact which tends to support Dr. Bishop's previ-

ous contention that the spermatozoa may be forced to depend largely on their intracellular phospholipid reserve during their transit of the female tract.

In collaboration with Dr. Seegar-Jones and Dr. John Woods, the spermicidal effects of vaginal cultures of the fungus *Candida krusei* have been explored in women and in monkeys. The survival of human spermatozoa in the vagina and in the cervix is impaired by the fungus, but monkey spermatozoa, generally trapped in the vaginal plug, apparently are not affected. Pregnancies resulted in monkeys having good cultures of the fungus in the vagina at the time of copulation. The results have been prepared for publication.

A joint paper with Dr. Albert Tyler, now in press, summarizes work on the demonstration and characterization of mammalian fertilizin in several animals. "Egg substance" given off by newly ovulated eggs activates and agglutinates spermatozoa of the same species, as in many invertebrates. The reaction in mammals shows some species specificity. In the rabbit and mouse it is characteristic only of recently ovulated eggs. Young follicular ova and old eggs surrounded by an albuminous layer do not react with sperm suspensions in this way. The results suggest that in mammals a type of serological mechanism is involved in the sperm-ovum interreactions in the early stages of fertilization.

### THE CARNEGIE COLLECTION OF EMBRYOS

During the year Mr. O. O. Heard with the aid of Mr. Duncan sectioned 18 human embryos ranging from the stage of 17 somites to 18 mm crown-rump length. Orientation photographs were made of all these; that is to say, each section was recorded in its exact relation to the embryo by photographing the top of the paraffin-celloidin block as the sections were taken off by the knife. In the preparation of these 18 specimens, 9464 individual photographs were thus made on strips of film,

one for each section that was cut and mounted. Two of these embryos (nos. 8505a and 8505b) are a pair of dichorionic (fraternal) twins at the 22-somite stage. Four pairs of embryonic temporal bones were also sectioned for study of the internal ear by Dr. Bartelmez.

Three very unusual specimens illustrating the development of other primates were successfully sectioned. One of these is an undivided egg cell of the rhesus monkey with polar body, sectioned serially

at 4 microns. The others were two embryos of the primitive primate *Tarsius spectrum*, respectively 8 mm (no. 290C) and 4 mm (no. 433C) in length. These rare and, from the evolutionary standpoint, exceedingly interesting embryos were among the preserved specimens received with the Bluntschli Collection (see Year Book No. 50, 1950-1951). The records show that they were given to Professor Bluntschli by the Hubrecht Laboratory of Utrecht. They were collected for the late Professor A. W. Hubrecht on the island of Banka (Indonesia) in 1894 and 1895, and had therefore been preserved in alcohol for about sixty years, yet when stained and mounted their tissues were found to be in excellent condition for microscopic study.

Dr. Ramsey and Miss Caspari continued the arduous work of culling, from the specimens stored in bottles and jars, those which have no research or museum value. The screening of current additions has been improved, so that only good and

useful material is stored, and a program of semiautomatic review will be instituted.

An interesting side light on the collection of human embryos appeared as the result of a search for all recorded embryos having 4 to 12 somites. This search was made in connection with the preparation of a forthcoming chapter (horizon x) of the series "Developmental Horizons in Human Embryos," begun by the late Director, G. L. Streeter. In all, records were found of 32 embryos of that stage known to science. Of these, 15 are in the Carnegie Collection, and 4 others are represented there by tracings, photographs, or models. Thus more than half the world's material for this relatively inaccessible phase of human development is available for study at the Department of Embryology. No other collection includes more than 3 embryos of this stage. The larger the number that can be readily compared, the more certainly can a standard of normal development be established.

## PUBLISHED RESEARCH

### PATHOLOGY OF EMBRYO AND FETUS

*Prenatal mortality.* A chapter by Dr. Corner and Dr. Bartelmez in the Pregnancy Wastage Symposium volume (see bibliography) is a brief preliminary version of an article now in press (Contributions to Embryology, vol. 35) describing nine cases of embryonic abnormality and death in rhesus monkeys, occurring in the first two weeks of pregnancy. This work was mentioned in Year Book No. 49 (1949-1950) under "Program of Investigations." The essential finding is that in some of the cases the embryonic abnormality probably resulted from constitutional defects of the embryo itself. In others, primary failure of the corpus luteum may have occurred.

*Harelip and cleft palate.* Congenital defects of the upper lip and the palate confront the embryologist with a particularly distressing problem. About one child in

700 is born with such defects in greater or less degree. The failure of development which produces them occurs at about the 6th week of embryonic life. Material for embryological study is however scarce, only four early specimens having been reported. When Dr. Richard B. Stark, a plastic surgeon of New York, undertook to restudy the problem, the Department of Embryology was able to put at his disposal four more specimens, to which he added another acquired in New York.

In brief, there are two hypothetical explanations of these defects. One, the "classic theory," explains them by supposed defective fusion of processes which grow together from the two sides of the embryonic facial region to form the upper lip and premaxilla. The other, more recent, concept is that the epithelial tissues which cover the lip and palate form and fuse but are not invaded by embryonic connective



tissue (mesoderm). The epithelial wall then thins and pulls apart, with resultant harelip or cleft palate or both.

Dr. Stark sectioned the five specimens at his disposal and measured the amount of mesoderm in the normal and defective regions of the face. He concludes that the lip and premaxilla pass through a stage in which three masses of mesoderm are present within the epithelial structures. These normally grow and unite to form the supporting layers of the region. If one mass of mesoderm is missing, the epithelium is pulled apart and a cleft results, its location depending on whether the missing mass is lateral (unilateral cleft) or medial (median cleft). If both lateral masses are missing, bilateral harelip results. In the region of the hard and soft palate Dr. Stark did not find evidence for a failure of mesodermal development; this fact suggests that with regard to the palate the "classic theory" is correct.

*A unique anomaly.* Several years ago Dr. Charles Posner of New York asked the Department's help in the study of a very peculiarly malformed fetus which had been delivered in his obstetrical service at the Bronx Hospital. The most striking element in the monstrosity was a proboscis-like snout in place of the mouth and nose. No similar case could be found in the scientific literature, although Dr. Corner found in the Sir Jonathan Hutchinson Collection of medical pictures in the Welch Library of Johns Hopkins Medical School an early nineteenth-century broadside of a "pig-faced lady" who seems to have had a similar though less extensive deformity. The anatomical part of Dr. Posner's published account is based on a dissection done by Mr. Barry Plunkett at the Department of Embryology and illustrated by a photograph taken there. The specimen and notes are filed as no. 8747 of the Carnegie Collection.

#### NERVES OF THE UTERUS

As was mentioned in Year Book No. 52, Dr. Corner, while at Oxford as George

Eastman Visiting Professor, 1952-1953, joined with Dr. Graham Weddell and Dr. Wazir Pallie of the Oxford Department of Anatomy in a study of the intrinsic nerves of the uterus. The part played in uterine function by the nerves supplying the musculature of that organ is obscure. The motility of the organ is largely automatic and yet can be influenced by factors that operate through the nervous system. The study made at Oxford was done on the rabbit, because that animal has been extensively utilized in experimental work on uterine function. The most advanced technique of intra-vitam staining of the nerves with methylene blue, developed by Dr. Weddell and his colleagues, was applied. The uterus proved to be very difficult to stain, a fact which explains the sparseness of information previously available.

The picture of the intrinsic nerves of the rabbit's uterus thus revealed is much richer in detail than has been described by previous workers. The nerve supply consists of a plexus of myelinated and unmyelinated fibers. From this, single fibers are given off which branch and mostly end as finely pointed twigs between the muscle cells. Some of the twigs end in small terminal enlargements. The nerve endings, although closely approximated to the muscle elements, do not have a one-to-one relation to individual muscle cells like that of the end plates of skeletal muscle. The terminations are not sufficiently numerous to provide each uterine muscle cell with a nerve ending. The blood vessels in the myometrium are accompanied by unmyelinated nerves with terminations similar to those of the myometrium. No ganglion cells and no specific sensory endings are found in the uterine horns.

In structure, and presumably in function, the innervation of the myometrium resembles that of the blood vessels. It is suggested that this very primitive type of nerve supply is adequate to mediate the neural control of the uterine muscle, which (so far as understood at present) is broadly co-ordinative, slow-acting, and not sharply

localized. Innervation of this type is not incompatible with the known domination of the ovarian hormones over the specific pattern of motor response to drugs and to stimulation by way of the nerves.

#### PLACENTAL CIRCULATION

In March 1953 Dr. Ramsey presented to the Brooklyn Gynecological Society a preliminary account of the studies of the circulation in the maternal placenta of primates, in the form of a lecture which has since been published. This was based largely on the rhesus monkey. A detailed and fully illustrated article is in press for the forthcoming volume 35 of *Contributions to Embryology*. She finds that during the period of growth, the endometrial arteries are lengthened and become increasingly coiled. In the phase of stretching of the pregnant uterus, the coils are paid out. Endometrial veins grow less extensively than do the arteries, and adapt themselves to both phases of uterine enlargement largely by passive alterations in caliber. The marginal sinus is constantly recognizable after the 6th week of pregnancy. Its drainage is largely indirect. Basal drainage channels persist to term and are responsible for at least half of the venous drainage of the placenta. Overflow filling of the placenta is achieved by the force of the maternal arterial blood pressure, which exceeds the pressure in the intervillous space by 60 to 70 mm Hg, rather than by the action of septal dividers. A similar study of the human placenta is mentioned above under "Research in Progress."

#### PHYSIOLOGY OF THE FETAL CIRCULATION

Three papers by Dr. Reynolds, listed in the appended bibliography, report work that was fully summarized in Year Book No. 52 under "Research in Progress." These deal with the regulation of the fetal blood pressure and heart rate, as investigated in experiments on pregnant ewes. The general conclusion from this work is

that the reflex mechanisms of cardiac control, operative in postnatal life, do not ordinarily function in utero. When, however, the fetus is submitted to conditions of stress, the cardiac nerves, both sympathetic (accelerator) and vagal (depressor), go into action, and moreover the adrenal cortex secretes adrenaline to stimulate the heart and raise the blood pressure. The mechanism is ready even though in the normal protected situation of the fetus it is not called into use. Under the non-stressed conditions of intrauterine life there is, however, some sort of internal regulation of the heart rate, under the control of the blood pressure by mechanisms which remain to be elucidated.

A fourth paper published during the year by Dr. Reynolds has also been summarized in Year Book No. 52. In this article the question of the source of the amniotic fluid is raised, and the suggestion is made, on the basis of direct observations, that the nasopharynx and buccal cavity of the fetus may (in the sheep at least) be a significant source of this fluid.

#### BIOCHEMISTRY OF THE FETAL LIVER

*Oxidative enzymes.* From the laboratories of Dr. Louis B. Flexner, Research Associate of the Department of Embryology, now at the University of Pennsylvania, papers continue to appear reporting progress in his program, begun in Baltimore, of studying the development of function in the fetal liver and brain in comparison with the structural development of those organs. An article by Dr. Flexner, Dr. E. L. Belknap, and Dr. Josefa B. Flexner deals with two respiratory enzymes, succinic dehydrogenase and cytochrome oxidase. Both these physiologically essential active substances are found to be present in the guinea-pig brain in low concentration until about the 40th day, and then to increase rapidly. The period of the 40th to the 45th day of fetal life (term 66 days) has previously been found to be one of profound change in anatomical structure of



the brain tissues, and also in biochemical activity. In the liver, cytochrome oxidase increases progressively without evidence of a critical period; succinic dehydrogenase remains low until about the 60th day and then rises rapidly.

*Glycogenesis.* Dr. A. M. Nemeth, Dr. William Insull, Jr., and Dr. Flexner have recently reported the results of a study of the development of glycogenesis in the fetal liver. In the guinea pig, glycogen is not demonstrable by chemical means until the 57th day of gestation, although it is present in the skeletal muscle and heart muscle at least as early as the 39th day. A search was made in the liver, before glycogen appears, for the enzymes and substrates known to be responsible for the synthesis of glycogen from glucose. Evidence of the presence of all these was obtained, i.e. hexokinase, phosphoglucose mutase, glucose-1,6-diphosphate, phosphorylase, polysaccharide (serving to prime the phosphorylase reaction), and "branching enzyme." The tentative conclusion is that the absence of glycogen in the liver before the 57th day of gestation is due to an inadequate number of end groups in the polysaccharide primer; this may reflect a relative deficiency of "branching enzyme" activity.

#### PHYSIOLOGY OF UTERINE MUSCLE

*Intracellular potassium and sodium.* On the basis of experiments which have been reported in previous Year Books, Dr. Csapo proposed a hypothesis to explain the difference in the behavior of uterine muscle when dominated by the ovarian hormones estrogen and progesterone, respectively. This hypothesis assumes that intracellular potassium is involved in the contraction pattern, and that under progesterone domination the access of potassium to the inside of the cell is retarded, and its ability to contract is thus limited. To test this hypothesis it was necessary to study the intracellular potassium-sodium ratio directly. This has now been done by

Dr. Beni Horvath, using the flame photometer after appropriate preliminary treatment of the muscle samples. The extracellular water in the muscle was estimated by the inulin method, and also by calculating sodium and chlorine spaces by measuring the amounts of sodium and chlorine given off by a known weight of muscle to a known volume of glucose solution. The results indicate, in accord with the hypothesis, that progesterone-dominated uterine muscle has a higher concentration of sodium and a lower concentration of potassium than does estrogen-dominated muscle.

*Ionic gradients (potassium and sodium).* From the studies on potassium and sodium concentration in relation to muscle contraction, Dr. Csapo has arrived at important conclusions which have been published in the English journal *Nature* in a closely knit article which is difficult to summarize here. Briefly put, when muscular tissue contracts, two processes are involved. One is the actual shortening of the contractile protein complex actomyosin. The other is the activation or initiation of the contraction, which can be elicited by electrical stimulation, by certain drugs, by sharp temperature changes, and by nerve impulses. To produce a contraction of living muscle tissue in vitro there must be present (1) actomyosin; (2) adenosine triphosphate to supply energy; (3) a proper proportioning of the concentration of potassium and sodium within and without the muscle cells. Dr. Csapo finds that he can separate the contraction and the activation by raising the proportion of the potassium ions in the bath in which his muscle strips are suspended. This results in increased potassium concentration within the cells, and therefore abolishes the difference or gradient between the interior of the cell and its environment. In this condition the muscle becomes inexcitable, developing little or no tension when electrically stimulated at a voltage which normally initiates effective contraction. The basic or "final" contractile mechanism re-

mains intact, and can be activated by a very much stronger electrical stimulus, which is thought to act directly on the contractile protein.

The characteristic phenomena of the uterus described by Csapo and Corner in 1952, namely positive and negative staircases under estrogen and progesterone domination respectively, are absent when the ionic gradient is abolished; this fact indicates that the staircase phenomenon is related to the process of activation rather than of contraction. Other implications of his findings are briefly discussed by Dr. Csapo.

*Isometric tension.* An article by Dr. Csapo in the *American Journal of Physiology* reports work already summarized in full in Year Book No. 52 under "Research in Progress." In brief, the conclusion is that the behavior of uterine muscle in vitro must be studied by recording and measuring its contractions under isometric conditions, in which it can contract with maximum efficiency, rather than under isotonic conditions, which do not give maximum efficiency except under special conditions. Quantitative physiological studies, and pharmacological tests of drugs commonly employed as oxytocic agents, can be conducted accurately only if the considerations expounded in this paper are applied in the experiments.

#### ENDOMETRIOSIS

The Department of Embryology has been deeply interested in an experimental study of endometriosis by Dr. R. W. Te Linde and Dr. L. R. Wharton, Jr., of Johns Hopkins Medical School, and Dr. Roger B. Scott of Western Reserve University. Housing for most of the monkeys used by this group has been provided by the Department, together with a good deal of technical assistance. One monkey belonging to the Department which developed endometriosis spontaneously was turned over for study and finally for autopsy. A second report of the work has recently been published. The results of an extensive

and varied series of experimental procedures continue to indicate that external endometriosis (i.e. in the peritoneal cavity and other extra-uterine sites such as laparotomy scars) may originate by implantation and growth of endometrial fragments shed by way of the tubes at the time of menstruation. Its spread may be due to implantation and growth of the abnormal tissue thus initiated, and to dissemination through blood and lymph vessels.

#### SURFACE FORCES IN MICROTOMY

One of the major necessities in embryological study is to obtain sections that are as free as possible from distortion. Compression by the knife as the sections are cut, in paraffin or paraffin-celloidin blocks, is a frequent source of distortion. Mr. O. O. Heard, who has been studying the mechanism of compression, finds that chips of paraffin adhere in thin layers to the facets of the steel knife, increasing the coefficient of friction and causing seizure between the cutting surface and that of the paraffin block, with resultant deformation. Mr. Heard attributes this difficulty to physical forces associated with the existence of a "Beilby layer" on the polished facet. Sir George Beilby (1921) showed that in the polishing of a polycrystalline substance an amorphous layer is produced having properties different from those of the underlying crystalline material. Mr. Heard finds that the polished facets of a microtome knife are in fact clad with a Beilby layer. By etching the surface with fine abrasives he has been able to diminish its tendency to hold and drag the paraffin. A more practical method for actual use in section cutting is to coat the Beilby surface with a chemical substance that will alter the surface characteristics and thus reduce the coefficient of friction without altering the cutting edge as much as does etching. This has been done successfully by forming a coating of cuprous and stannous sulfides on the knife after grinding and polishing. After such treatment, compression is measurably reduced.



## DIFFUSION AND POPULARIZATION OF RESEARCH RESULTS

*Lectures and conferences.* Dr. Corner was chairman of the International Committee on Anatomical Nomenclature at its meeting held at London, June 1 to 5, 1954, and while in England for this purpose led an evening conference at the Ciba Foundation, London, on the anatomy and physiology of the two-horned uterus.

Dr. Burns lectured on the hormonal control of sex differentiation, at Winthrop College, Rock Hill, South Carolina; Emory University, Atlanta, Georgia; the Biological Station, Mountain Lake, Virginia; and the Department of Biology, Syracuse University, New York. He also gave a seminar at the Department of Anatomy, University of Rochester, on transformation of the testis induced by sex hormones.

At the invitation of Dr. Milislav Demerec, Dr. Reynolds was chairman of the Nineteenth Cold Spring Harbor Symposium on Quantitative Biology, June 7 to 14, 1954. Dr. Reynolds devoted a great deal of time to the organization of the symposium, in which the Department of Embryology was represented by Dr. David Bishop, Dr. B. G. Böving, Dr. R. K. Burns, Dr. Elizabeth M. Ramsey, and Dr. A. St. G. Huggett (guest investigator), all of whom participated by presenting or discussing papers.

Dr. Reynolds gave the Bacon Lecture at the University of Illinois in January 1954, and the Annual Lecture of the Washington (D. C.) Gynecological Society in May and of the Chicago Gynecological Society in June 1954. He also assisted in the organization and served as chairman of the Second Conference on Congenital Anomalies held by the Association for Aid to Crippled Children, led three conferences at Johns Hopkins Hospital (Departments of Surgery, Anesthesia, and Pediatrics), and presented lectures and talks before about a dozen other medical and scientific organizations in various parts of the country.

Dr. Ramsey lectured on the embryology of the female reproductive tract at the

George Washington University Post-Graduate Course in Obstetrics and Gynecology (March 1954); on obstetrical and gynecological pathology in her capacity as Professorial Lecturer at the same School (April 1954); and on the placental circulation at Sinai Hospital, Baltimore (March 1954). She took part in a panel discussion of biological aspects of mental deficiency, held by the National Association for Retarded Children at New York in June 1954.

*Reviews and general articles.* Dr. Reynolds published three review articles during the year. One of these, in the *Scientific Monthly*, deals with circulatory adaptations to birth, and is based chiefly on the work done by Dr. Reynolds with a group at Oxford in 1951-1952. At the First World Congress on Fertility and Sterility, held at New York in May 1953, he presented a plea for research on uterine function as a basis for control of fertility. To a gynecological journal published at Ludhiana, an important medical center in India, he contributed by invitation a review of knowledge concerning the physiology of the cervix uteri.

A chapter by Dr. Csapo in the Pregnancy Wastage Symposium is, in effect, a brief review of his work on the biochemistry and physiology of uterine muscle up to the time of the Symposium (1951) and a plea for study of these topics as a basis for understanding and controlling prenatal mortality due to malfunction of the myometrium.

Dr. Bishop prepared a paper on sperm maturation for the *Scientific Monthly*, and a chapter on the biology of spermatozoa for the forthcoming third edition of the handbook *Sex and Internal Secretions*, for which Dr. Burns also prepared a chapter as previously reported.

*Photography.* Mr. Richard D. Grill, Photographer, received an award of merit for work exhibited at the Twenty-third Annual Meeting of the Biological Photographic Society, Los Angeles, September

1953. He was also awarded a blue ribbon for pictures of human embryos shown at the Ninth Chicago International Exhibition of Nature Photography, Chicago Natural History Museum, February 1954.

*Visiting groups.* Tours of the laboratory, with suitable demonstrations, were arranged for the science classes of the Samuel Ready School and the Roland Park Country School of Baltimore.

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## DEPARTMENT OF GENETICS

*Cold Spring Harbor, Long Island, New York*

M. DEMEREC, *Director*

Early this year the research activities of the staff members of this Department were transferred from the old laboratories into the modern and well equipped laboratories of our new buildings. The time lost in this transfer has been more than offset by the greater efficiency of the new quarters. Good progress has been made during the year in converting the old Main Building into a library, where all our books will be readily available and where study rooms and a small conference room will be conveniently located. We have also made good progress in modernizing the old laboratories of the Animal Building for use by guest scientists, and in landscaping our grounds to conform with the new buildings.

### RESEARCH

In the course of the year McClintock has continued her studies of mutation in maize at Cold Spring Harbor and carried out examinations of chromosomes of *Neurospora* at the California Institute of Technology. The maize studies provided further information about the spread of mutation to either side of the locus occupied by the transposable chromosomal unit *Ds*. Attention was given mainly to seven independently induced cases in which the spread covered a relatively long segment to the left of *Ds*. All seven cases had in common certain types of change in genic action, suggesting that similar modifications had occurred in all of them within the same chromosomal region. The differences among them suggested different extents of change beyond this region. To gain a fuller understanding of the kinds of systems in the maize nucleus that can control genic action or gene mutation, intensive study was begun of a system showing a type of action not previously

encountered. The investigations with *Neurospora* were made in order to determine the nature of chromosomal aberrations known to be present in several strains. Unique kinds of meiotic behavior of chromosomes were noted, as well as unanticipated types of structural modification. The findings of this study demonstrated very clearly that chromosome organization and behavior in different genetic strains of *Neurospora* should be investigated, and that, in many cases, such knowledge should be available before interpretations are drawn from genetic evidence.

Cytochemical studies using purified enzymes have been continued by Kaufmann, McDonald, and their group. As reported last year, immersion of growing roots in solutions of ribonuclease produces the types of aberration that are known as "primary effects" when they are induced by ionizing radiations. No chromosome breaks or structural rearrangements were found by Kaufmann and Kersh in studies of the effects of an aerosol of ribonuclease on spermatozoa of *Drosophila*. It is suggested, therefore, that primary effects can be induced by degradation of structural ribonucleoproteins, whereas secondary effects (structural rearrangements) involve more profound degradation of the nucleoprotein complex.

The validity of interpretations derived from studies using enzymes depends on the specificity of action of the enzymes. McDonald and Kaufmann have recently examined another case of reported non-specificity of action, namely, the degradation of apurinic acid (deoxyribonucleic acid from which the purine bases have been removed) by ribonuclease. They have shown that the ability to degrade apurinic acid is not a specific property of



ribonuclease. Similar results are obtained with lysozyme, cytochrome c, histone, chymotrypsin, chymotrypsinogen, and trypsin, but not with egg albumin or serum albumin. The degradation of apurinic acid by these proteins is a function of their basicity, the most basic ones being the most effective. The rate of degradation is dependent on temperature and pH, and is proportional to the concentration of native protein in the reaction mixture. The extent of degradation, however, is independent of the protein concentration.

Another aspect of this problem was examined by Kaufmann and Ghosh, who found that cytochrome c reduces pyronin stainability in tissue sections, as if the enzyme were degrading ribonucleic acid. Preliminary analysis suggests, however, that the result is due to a combination of the enzyme with ribonucleic acid because of its action as a basic protein. When used in treatment of growing roots, cytochrome c induces excessive contraction and stickiness of chromosomes, some *c*-mitoses, and polyploid chromosome complexes.

McDonald and Ghee have continued their investigations of intracellular deoxyribonucleases. These enzymes differ both functionally and chemically from the deoxyribonuclease secreted by the pancreas. Only the latter, whose function is primarily digestive, has been purified and crystallized. Methods are now being developed for the purification of calf-spleen deoxyribonuclease, since the exact role played by the intracellular deoxyribonucleases in cell division cannot be established until they have been isolated and characterized.

Gels of materials of nuclear origin were exposed by Bernstein to 1000 r of X-rays, and viscosity measurements were made. There is no difference in viscosity between irradiated and nonirradiated gels immediately after treatment, but after storage for a few hours the viscosity of the irradiated gels is markedly less than that of the controls. These and related studies suggest that the radiation-sensitive structural ele-

ments of these gels are the secondary intermolecular associations between nucleoprotein molecules.

Development by Gay of a method for obtaining ultrathin serial sections for electron microscopy, in any quantity desired, has enormously improved the prospects of formulating interpretations of three-dimensional patterns of cellular organization. The disclosure of structural detail afforded by this technique has already opened up new vistas for the analysis of nuclear-cytoplasmic relations. Another technique developed by this worker for the imbedding and sectioning of salivary-gland-chromosome smears offers the opportunity for electron microscope study of specific chromosomal regions with known genetic properties.

Hershey's group has continued to investigate the role of nucleic acid in viral inheritance. They find that the transfer of labeled DNA from parental to offspring phage cannot be influenced by adding non-radioactive nucleosides to the culture medium as competitors. This means that the DNA is transferred as relatively large pieces, and therefore conceivably as genetically functional pieces. The conversion of bacterial to viral nucleic acid in infected bacteria has been studied by similar methods. Evidence of breakdown en route was obtained, but here too it appears that the intermediates must be relatively complex materials. This result is perhaps significant in connection with the many other evidences of interaction between virus and bacterial nucleus.

Garen has shown that in mixed infection with T2 and T4 bacteriophage, when only the T4 DNA is labeled with radioactive phosphorus, about 35 per cent of the transferred radioactivity appears in T2 progeny. This might indicate genetically nonspecific transfer or material exchange during genetic recombination.

Hershey and Hudis have made a descriptive analysis of protein synthesis in infected bacteria. They find that infection does not interrupt the synthesis of bacterial

protein. Rather, the synthesis of viral protein only gradually and partially replaces the synthesis of bacterial protein, with little diminution in rate. The amount of viral-precursor protein in the infected cell is appreciably less than the amount of viral-precursor nucleic acid, a fact which suggests that most of the protein for a given virus particle is formed after its nucleic acid has already been laid down.

Dorothy Fraser has found that a large class of "mutants" of phage T<sub>3</sub> arise only in bacteria in which lysis is delayed. The changes responsible for this class are clearly distinct from viral mutations of the usual type, which occur with characteristic frequencies independently of the condition of the host cell. The kinds and frequencies of the new class of changes depends not on the bacterium and the phage singly, but on the relation between them. She is pursuing the hypothesis that the underlying changes are not primary modifications of the genetic material of the phage, but result from exchanges of material between bacterium and phage.

Witkin and Lacy have initiated a study of the kinetics of transduction in *Salmonella typhimurium*. They have shown that transductions, like induced mutations, are characterized by a pattern of delayed appearance as a function of the multiplication of the treated population, which is stable and specific for different loci. Their results suggest that the degree of dominance exhibited by the locus in question may affect the pattern of delay. It was found that transduced cells do not occur in pure clones, but in mixed clones containing also nontransduced cells of the parental type. These results are compatible with two hypotheses concerning the mechanism of incorporation of genetic material carried by transducing phage: (1) incorporation into the new bacterial chromosome during multiplication; (2) incorporation by replacement of the homologue before replication in one of the strands in a multiple-strand chromosome. Witkin and Lacy have also carried forward

the study of the delayed appearance of ultraviolet-induced mutants, using transduction as an experimental tool to narrow down the list of possible factors involved. They have eliminated a series of previous postulates, and have established that the major factors are those arising from the unique behavior of newly induced mutant cells, namely, delayed mutation or selectively delayed onset of multiplication, or both.

Further elucidation of the mechanism of transduction in *Salmonella* has been provided by the work of Demerec and Blomstrand. The results of their experiments are consistent with the assumption that a replica of the transducing segment, formed during chromosomal duplication, is incorporated into the newly duplicated bacterial chromosome by a process similar to the mechanism responsible for crossing over in higher organisms. These experiments also suggest that the length of the transducing segment is only a small fraction of the length of the bacterial chromosomes.

Studies have been made by Demerec, Blomstrand, Brenner, Zlata Demerec, Hartman, Nielsen, and Yura of a number of *Salmonella* strains deficient in the synthesis of adenine, cystine, galactose, histidine, proline, serine, or tryptophane. It has been shown that similar auxotrophs (those carrying the same deficiency) can be separated by transduction tests into well defined groups, and that this grouping coincides with that obtained by certain biochemical methods which investigate blocks in the reaction chains leading to the synthesis of the compounds required by the auxotrophs. These findings suggest that each group represents alleles of the same locus, that a gene locus extends over a section of chromosome, and that changes occurring in different regions of this section give rise to different alleles. They also indicate that regions within a section may separate, and recombine with homologous regions within a locus of another chromosome.

Investigations carried out by Yura have



shown that suppressor mutations in *Salmonella* can be detected and analyzed genetically by transduction studies involving the mutant gene and its suppressors.

Spontaneous mutation in steady-state populations of B strains of *Escherichia coli* has been investigated by Moser with the aid of the chemostat. In the strains studied, the accumulation with time of T5-resistant mutants and revertants from nutritional requirement proved to be a curvilinear one, indicating selective advantage or disadvantage of the mutant in the growth tube of the chemostat. A form of mathematical analysis was developed which makes it possible to determine spontaneous-mutation rates at different rates of growth when selection operates either for or against the bacterial mutant. Applying this method to strain W-74, he was able to observe an effect of growth rate upon mutation rate per unit time in cultures in which nitrogen was used as the controlling growth factor. At low rates of growth, up to a generation time of about 3.5 hours, mutations to phage resistance occur at a constant rate of about  $1.6 \times 10^{-8}$  per hour per cell. With shorter generation times, however, the mutation rate per unit time increases significantly with increasing growth rate.

Comparative studies of the action of various mutagens on certain genes of *E. coli* have been continued by Glover, using ultraviolet rays, X-rays, nitrogen mustard, diepoxybutane, and triazine. In addition to confirming the earlier finding that the mutability of genes is affected to different degrees by different mutagens, he observed several cases of specific mutagen stability—demonstrating that the mutability of some genes is increased by treatment with certain mutagens but remains unaffected by treatment with others. He also found that both spontaneous and induced mutability in certain genes is related to the genetic constitution of the bacterial strain.

Modification of the biological, and particularly the genetic, effects of ultraviolet

radiation by various environmental factors has been studied by FitzGerald. The results of her experiments support the following hypotheses: that ultraviolet treatment induces in cells certain changes that affect their metabolism, which in turn influences both vital functions (survival) and genome (mutability); that for some time after irradiation the cell is in an unstable state, during which time the environment can influence the course of events leading to the final state; and that peroxides are involved in one of the ultraviolet-induced reactions leading to the continuing after-effects observed.

Twenty-seven compounds of interest in connection with cancer chemotherapy have been investigated for mutagenicity by Hemmerly. Table II of this report summarizes the results of her experiments.

#### STAFF

Evelyn M. Witkin, who was on leave of absence during 1952–1953, returned to work on a half-time basis this year. Barbara McClintock spent the period from November to March at the Biology Division of the California Institute of Technology in Pasadena, making a cytological analysis of *Neurospora* chromosomes and giving a series of lectures on her research with maize.

The Department had two fellows: Alan Garen, working for the second year with Hershey under a fellowship of The National Foundation for Infantile Paralysis; and Hermann Moser, a Carnegie Institution Fellow, who worked in Demerec's laboratory. Dr. Sydney Brenner, of the University of the Witwatersrand, Johannesburg, South Africa, in this country as a Fellow of the Carnegie Corporation of New York, was with us for about three months doing research in bacterial genetics. During the summer of 1954 Dr. Joseph S. Gots of the University of Pennsylvania spent six weeks as a guest of the Department, carrying out biochemical analyses of bacterial mutants.

## CO-OPERATIVE WORK

We have continued to work in close co-operation with the members of the Biological Laboratory of the Long Island Biological Association. The seven members of the Laboratory research staff took part in our weekly staff meetings; and they and their assistants attended our regular seminar lectures and literature-review meetings. A major portion of the year-round research at the Biological Laboratory deals with genetical problems related to those studied at this Department. V. Bryson, W. T. Szybalski, P. D. Skaar, and J. Q. Heplar worked during the past year in the field of bacterial genetics; and B. Wallace and J. C. King continued their studies of population genetics with *Drosophila*.

Our staff members profited also by association with other research workers who stayed at the Department and at the Biological Laboratory for various periods during the year. Among these were: Mark Adams, of the New York University College of Medicine; S. Benzer, of Purdue University; F. H. C. Crick, of the Cavendish Laboratory, Cambridge, England; S. Granick and R. D. Hotchkiss, of The Rockefeller Institute for Medical Research; F. Kaudewitz, of the Max-Planck-Institut für Virusforschung, Tübingen, Germany; David Shemin, of the College of Physicians and Surgeons, Columbia University; G. S. Stent, of the University of California, Berkeley; Leo Szilard, of the Institute of Radiobiology and Biophysics, University of Chicago; J. D. Watson, of California Institute of Technology; and N. Zinder, of The Rockefeller Institute for Medical Research.

Members of our staff have co-operated in various ways with scientists at other institutions, particularly members of the Brookhaven National Laboratory at Upton, New York. Kaufmann collaborated with Dr. M. J. Moses, Mr. W. A. Higinbotham, and Mr. R. Chase of that Laboratory in adapting the RCA television camera for use in

cytological research; and McClintock utilized facilities provided by Dr. W. R. Singleton for drying her experimental corn. Kaufmann also continued to co-operate with Dr. T. F. Anderson, of the Johnson Foundation of the University of Pennsylvania, and with Dr. Keith Porter, of the Rockefeller Institute, in electron microscope studies.

## MEETINGS AND LECTURES

The Nineteenth Cold Spring Harbor Symposium on Quantitative Biology, held in June 1954 under the auspices of the Biological Laboratory, was attended by more than a hundred scientists interested in physiological aspects of the development of the mammalian embryo. Twenty of the participants were from abroad. The program of this symposium was organized by Dr. S. R. M. Reynolds, of the Carnegie Institution's Department of Embryology.

In August 1954, a conference of scientists interested in research with bacterial viruses was held at the Biological Laboratory. It was organized by Hershey, and attended by about seventy participants.

Weekly meetings of the research staffs of the Department and the Biological Laboratory were held from October through May, for informal discussion of scientific problems of general interest and reports of the current research of the individual members. Seminar lectures also were held each week throughout most of the year. They were attended by the scientific members of the Department and the Biological Laboratory, and occasionally by scientists from near-by institutions. The speakers, who included members of the staff, members of the summer group at the Biological Laboratory, and invited guests, presented reviews of completed research problems in which they had made major contributions. During the winter and spring, short weekly meetings for review of current literature were attended regularly by all members of the scientific group.



## OTHER ACTIVITIES

The nineteenth issue of *Drosophila Information Service*, a mimeographed bulletin compiled from the contributions of *Drosophila* research workers throughout the world, was prepared at the Department under the editorship of Demerec. Witkin continued to compile and edit the *Microbial Genetics Bulletin*, issuing numbers 8, 9, and 10 of the *Bulletin* during the year covered by this report.

The *Drosophila* Stock Center, with Mrs. G. C. Smith as curator, sent out a total of 1239 cultures in response to requests from teaching and research laboratories. Sixty-three of these cultures were shipped abroad, to workers in Europe, Asia, and Australia.

The library, of which Mrs. H. H. Wheeler is librarian, acquired 81 books in the course of the year, and received 332 periodicals and serial publications by subscription, gift, or exchange.

## GROWTH AND INHERITANCE IN BACTERIOPHAGE

A. D. HERSHEY, ALAN GAREN, DOROTHY K. FRASER, AND JUNE DIXON HUDIS

Putnam and Kozloff first showed that isotopically labeled phage particles contribute some but not all of their labeled atoms to their progeny. Their work and that of their successors has given rise to the following well defined questions about the transferred atoms: (1) Origin—in random or special parts of the parental particles (Putnam and Kozloff)? (2) Location—in random or special parts of the offspring particles (Maaløe and Watson)? (3) Route—by way of large specific pieces or small nonspecific ones (Kozloff)? (4) Distribution—in one, several, or all the offspring particles (Hershey, Kamen, Kennedy, and Gest)? (5) Function—transferred independently, or not, of the transfer of genes (Kozloff)?

Questions (1) and (2) were partly answered by Hershey and Chase, and by French, who found that only the viral nucleic acid (DNA) contributes and receives conserved atoms. Question (1) remains unanswered with respect to parts of the DNA, however, because only about 40 per cent of its label is transferred. Question (2) was further answered by Maaløe and Watson, who showed that the DNA of the offspring of labeled particles is itself randomly labeled with respect to transfer during a second cycle of growth. Question (4) was partly answered by Watson and Maaløe, and by French, Graham, Lesley, and van Rooyen, who found that

only particles formed early receive parental atoms. As is described in this report, Garen has now shown that the transferred atoms originating from a single parental particle enter into several offspring particles. Most of the work to be summarized here, however, deals with attempts to answer questions (3) and (5).

Some of our experiments relate also to a question rather new to both genetics and virology. This involves the relation between virus and bacterial nucleus. Rather diverse lines of genetic evidence (Lederberg and Lederberg, Zinder, Bertani, Lwoff and his collaborators) show that some close relation exists in lysogenic bacteria. Dorothy Fraser has carried the idea of nuclear localization to its logical conclusion by looking for evidence of genetic recombination between phage and bacterium. In addition, we are exploring possible ways of bringing chemical methods to bear on the question, and some of our work with T2 has this objective.

As in the past, the work with bacteriophage was supported in part by a grant (C-2158) from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service. Isotopes were supplied by the Oak Ridge National Laboratory on allocation from the Atomic Energy Commission. Garen's participation in the work was made possible by his

fellowship from The National Foundation for Infantile Paralysis.

#### BIOCHEMICAL ROUTE FROM PARENT TO OFFSPRING PHAGE

The chemistry of the transfer of atoms from parental to offspring phage interests us for two reasons. First, the atoms are those of DNA, primarily or exclusively, which suggests that DNA has a genetic function in T2. Second, it seems likely that most of the viral DNA is genetically potent, because the amount in a single particle of T2 ( $2 \times 10^{-13}$  mg) is small as compared with the amount per nucleus in most cells. In fact, current radiobiological experiments (Stent, Doermann) seem to show that more than 20 per cent of it is genetic material, and that the ratio of genetic to nongenetic DNA remains constant as one goes to still smaller phages. For these reasons we feel justified in hoping that the conservation of materials during viral growth is a genetic problem, as well as a biochemical one.

The isotope-competition method offers one way of investigating the biochemical route from parental to progeny DNA. This approach is inseparable from the problem of mechanisms of synthesis of DNA, about which equally little is known. In this work we have benefited greatly from the generous advice of R. B. Roberts and E. T. Bolton of the Institution's Department of Terrestrial Magnetism, and from the active collaboration of A. F. Graham, L. Siminovitch, and S. M. Lesley at the University of Toronto, who first undertook experiments along the lines indicated here, and kindly permitted us to join them.

The experimental principle is as follows. Uniformly C14-labeled T2 is prepared by propagation in bacterial cultures containing radioactive glucose. The labeled virus is then allowed to multiply in cultures in which the parental viral material is the only source of C14. In these cultures the

source of food for the synthesis of new viral material is glucose, with or without added precursors of DNA such as guanine. Supplements of this kind are called competitors because they can be expected to compete specifically with intracellular sources of radioactive guanine and related substances. That they do so in fact is readily checked by placing them in competition with radioactive glucose.

Whether the source of radioactivity is the parental viral material or the glucose in the culture medium, the analytical method is the same. One isolates the viral DNA, hydrolyzes it to its constituent bases, separates these on paper chromatograms, and assays the radioactivity in the spots located by inspection in ultraviolet light. This method has the advantage that bases are recovered quantitatively, and the disadvantage that not all the radioactivity in the spots is contained in the bases. It is being used in conjunction with more precise methods worked out by the group in Toronto.

Table 1 summarizes the results of some isotope-competition experiments in which the parental phage was the source of radioactivity appearing in the offspring. In these experiments, 0.5 mg per ml of competitor was added at the time of infection. Phage yields were isolated after artificial lysis 1 hour later. They contained 30 to 50 per cent of the parental DNA C14. The results show that parental guanine, adenine, hydroxymethyl cytosine (hmc), and thymine are used equally to make the DNA of the offspring phage. Furthermore, this equal transfer cannot be upset by supplementing the culture medium with the competitors indicated. This must mean that the parental DNA is not broken down to the level of these intermediates en route to progeny DNA. Within the rather severe limitations of the method, the findings suggest that DNA is transferred in large pieces, and therefore conceivably in the form of genetically functional pieces.



TABLE 1  
TRANSFER OF C14 FROM PARENTAL TO PROGENY T2

| MATERIAL<br>ANALYZED             | PER CENT DISTRIBUTION OF C14 |     |         |         |
|----------------------------------|------------------------------|-----|---------|---------|
|                                  | Guanine                      | Hmc | Adenine | Thymine |
| Parental phage .....             | 18                           | 18  | 31      | 34      |
| Yield, no competitor .....       | 18                           | 17  | 30      | 35      |
| Yield, thymidine competing ..... | 18                           | 17  | 32      | 34      |
| Yield, guanine competing .....   | 18                           | 18  | 29      | 34      |
| Yield, adenine competing .....   | 18                           | 19  | 31      | 33      |
| Yield, thymine competing .....   | 18                           | 18  | 30      | 34      |

#### CORRELATION BETWEEN GENETIC AND MATERIAL TRANSFER OF VIRAL DEOXYRIBONUCLEIC ACID

A much more general clue to the meaning of the transfer of DNA from parents to offspring can be sought in terms of the correlation, or lack of it, between genetic and biochemical results. To this end we have studied the properties of phage inactivated by beta rays and ultraviolet light before infection, or self-inactivated after infection by attachment to previously infected bacteria. The principle of all the experiments is the same. One infects bacteria with two genetically and isotopically marked phages under conditions such that only one of them contributes genetic markers to the viral progeny. The latter are then examined to determine whether they contain isotope derived from the genetically excluded phage. Similar experiments have been performed before, by Kozloff and others. We have repeated them because we were dissatisfied with the results.

*Beta rays.* A preparation of T<sub>2</sub>, labeled with P<sub>32</sub>, was inactivated by suspending it for 10 days in a 2 per cent solution of peptone containing one mc per ml of P<sub>32</sub>. This exposure to beta rays killed 99 per cent of the phage, as determined by plaque titration. The particles were then washed free of external P<sub>32</sub> by centrifugation.

The inactivated phage was found to adsorb normally to bacteria, as measured by its radioactivity. When a suspension of cells with attached virus was spun in a

high-speed homogenizer (Hershey and Chase), 60 to 80 per cent of the radioactivity could be stripped from the cells. Only 20 per cent of the radioactivity of a sample of the same phage that had not been exposed to beta rays was removed under the same conditions. This shows that most of the inactivated particles do not inject their DNA into the cells to which they attach.

When the inactive phage was used to produce mixed infections with live, unlabeled phage, and samples of the culture were lysed at successive times by the method of Maaløe and Watson, it was found that most of the radioactivity sedimented either with the bacterial debris or with the small-particle fraction containing the viral progeny. The distribution between these two fractions was nearly equal, and was the same for lysates prepared before and after viral offspring appeared. This shows that the genetically excluded phage does not contribute detectable amounts of phosphorus to the progeny of the mixed infection. We suspect that the behavior of X-ray-inactivated phage will prove similar to that described above, and that the conclusions drawn from the earlier experiments of this kind are incorrect. On the other hand, the correlation between genetic and metabolic impotence of phage inactivated by ionizing radiations must be regarded as trivial, since the exclusion occurs outside the cell wall. Doermann, and Bertani and Weigle, have shown by ge-

netic experiments that a minority of the particles inactivated by ionizing radiations does contribute to the yield. Unfortunately, the limitations of the methods do not permit radiochemical analysis of this minority.

*Ultraviolet light.* When P<sub>32</sub>-labeled T<sub>2</sub> or T<sub>4</sub> is exposed to 150 times the average lethal dose of ultraviolet light, the subsequent ability to inject is only moderately diminished. This has been tested both by the blender experiment and by measuring the sensitivity of the viral DNA to deoxyribonuclease after adsorption of irradiated phage to heat-killed bacteria (Hershey and Chase). Apparently, ultraviolet light damages almost exclusively the injected material. The situation is a promising one, therefore, for the attempt to correlate genetic and metabolic potency. This attempt is all the more timely because Doermann is currently analyzing the genetic effects of ultraviolet light in quantitative terms.

Garen is making intensive efforts to measure the corresponding metabolic effects. He finds that the transfer of atoms from irradiated phage is not, as previously supposed, independent of radiation dosage. Whether the genetic and metabolic effects are directly correlated, however, is not yet clear.

*Superinfection.* When bacteria are infected with T<sub>2</sub>, and then reinfected 2 to 10 minutes later with P<sub>32</sub>-labeled T<sub>2</sub>, half the labeled DNA is quickly split into acid-soluble materials (Lesley, French, Graham, and van Rooyen). A negligible proportion of the DNA of the first particles to infect is split. Simultaneously with this effect, the superinfecting phage fails to contribute genetic markers to the viral yield produced by the first infecting particles (Dulbecco), and likewise fails to contribute labeled atoms to it (French *et al.*). Apparently the metabolic exclusion is primary, and the breakdown secondary, since streptomycin (which inhibits bacterial deoxyribonuclease) prevents the breakdown without affecting the exclusion. We have re-examined this situation, using a more spe-

cific method of inhibiting the bacterial nuclease, namely, low magnesium concentration.

We were interested in two questions. First, does the 50 per cent breakdown of superinfecting phage mean that viral DNA splits into two fractions having different properties? Second, does the viral DNA enter the cells when breakdown is prevented, and, if so, is it excluded from genetic and metabolic function?

The answer to the first question is no, because we find that only half the DNA of the superinfecting phage enters the cells, and practically all of this is broken down. If the blender experiment is performed using cells superinfected with P<sub>32</sub>-labeled T<sub>2</sub> in a medium containing  $10^{-3}$  M magnesium chloride, 50 per cent of the P<sub>32</sub> is excreted in acid-soluble form within 5 to 10 minutes, and 80 per cent of the remainder can be stripped away in the blender. If, instead, the medium contains  $10^{-5}$  M magnesium, less than 5 per cent breakdown occurs, and only 40 per cent of the P<sub>32</sub> is strippable. The low magnesium concentration does not itself affect injection, as is shown by similar experiments using P<sub>32</sub>-labeled phage for the primary infection.

At the low magnesium concentration, the 50 per cent of the superinfecting DNA that is injected remains in the cells until lysis. Phage growth is normal, and so is the transfer of P<sub>32</sub> from primary infecting phage to progeny. A repetition of Dulbecco's experiment under these conditions shows that the superinfecting phage makes no genetic contribution to the progeny. Similarly, P<sub>32</sub> contained in the DNA of the superinfecting phage does not appear in the progeny. These experiments show clearly that part of the DNA of superinfecting phage, although it enters the cells and is accessible to at least one bacterial enzyme, can persist there without metabolic function in viral growth. The correlation between genetic and biochemical results in this instance is encouraging, but not of great significance until more can



be learned about the mechanism of exclusion.

#### DISTRIBUTION OF TRANSFERRED ATOMS AMONG PROGENY

When a bacterium is mixedly infected with the related phages T2 and T4, progeny of both parental types are obtained. We were interested in determining whether, during mixed growth, the atoms contributed by one parent are located exclusively in its own progeny type, or in both types.

Garen examined this question in the following way. Stocks of T2 and T4 were first carried through several cycles of mixed growth in order to prepare "isogenic" strains, which could grow equally together under conditions of mixed infection. Bacteria were then mixedly infected with P32-labeled T4 and unlabeled T2 in buffer, and transferred for growth to a salt-free nutrient medium. The absence of salt prevented readsorption of phage after lysis. The total phage yield was 150 per cell and consisted of equal amounts of T2 and T4. The lysate was purified by treatment with deoxyribonuclease, followed by centrifugation at low and high speeds. In order to eliminate phenotypic mixing, the phage was put through a second growth cycle under conditions of single infection. Bacteria were infected in buffer with 0.3 particle per cell and then transferred to salt-free nutrient medium at a concentration of  $1.5 \times 10^8$  bacteria per ml. After 40 minutes' growth, sufficient for lysis of the infected bacteria, the culture was filtered. The yield in the filtrate was 160 particles per infected cell, and again it consisted of equal amounts of T2 and T4. The phage was purified by two cycles of alternate low- and high-speed centrifugation.

The T2 and T4 progeny from the second cycle of growth were separated by successive additions, first of B/2,4 bacteria to remove nonspecifically adsorbable material, then of B/4 to adsorb T2, and finally of B to adsorb T4. The results are given in

table 2. They show that P32 originally present in the parental T4 phage appeared not only in T4 progeny, but to a large extent in T2 as well. The results are in agreement with those of Hershey, Kamen, Kennedy, and Gest, who showed by a different kind of experiment that parental phosphorus atoms must be distributed to at least two progeny particles. At present it is not known what mechanism is responsible for the distribution of parental DNA, but one or both of the following are likely to be involved: transfer by way of genetically nonspecific units; material interchange during genetic recombination.

TABLE 2  
DISTRIBUTION OF P32 DERIVED FROM T4 AMONG  
PROGENY OF MIXED T2-T4 INFECTION

| Adsorbing<br>bacteria | Phage<br>adsorbed<br>(%) | P32<br>adsorbed<br>(%) |
|-----------------------|--------------------------|------------------------|
| B/2,4 .....           | .....                    | 5                      |
| B/4 .....             | T2: 92                   | 22                     |
| B .....               | T4: 85                   | 36                     |

#### THE ORIGIN OF HOST-RANGE MUTANTS OF T3

In work done by Fraser and Dulbecco at the California Institute of Technology, it was found that two host-range (*h*) mutants of the small bacteriophage T3 differed from the parent T3 in several loci, which showed segregation and recombination in crosses. Since the methods of isolation of these two *h* strains would normally be expected to provide single-factor mutants, it was of interest to discover why mutants differing from wild type in three or four factors were recovered instead. Fraser has pursued this question, with results suggesting that the mutants arise by interactions between phage and bacterium, possibly in the nature of genetic substitutions.

*The physiological conditions controlling mutation.* It has been found that a certain number of the host bacteria (*Escherichia*

*coli*, strain B) infected with T<sub>3</sub> fail to lyse at the end of the usual latent period, and that the progeny of such late-lysing bacteria contains host-range mutants, frequently comprising about 1 per cent of the yield, sometimes much more. Among the progeny issuing from cells that lyse promptly, no mutants are found within the limits of the method of detection (<1 in 30,000).

The proportion of such late-lysing bacteria, here referred to as complexes, depends on the physiological state of the bacteria before infection and on the treatment after infection. If the bacteria are starved by aeration in buffer for 2 hours before infection in broth, nearly all the bacteria form persistent complexes. If the culture is diluted in fresh broth at pH 6, the complexes begin to lyse after about 15 minutes. They are more stable in broth at pH 5.

Experiments have been carried out in which the infected bacteria are kept for periods of several hours to several days at 37° C in broth containing anti-T<sub>3</sub> serum to prevent further infection. A slow increase in the number of infected bacteria occurs under these conditions, indicating that the complexes are able to reproduce as such. It has not been possible, however, to obtain stable lysogenic clones. On dilution and plating, most of the bacteria lyse.

It seems likely that the complexes are the same as the cells of the "thin" colonies frequently found when phage T<sub>3</sub> and strain B are plated together for the isolation of resistants. Like the complexes, cells of thin colonies lyse on dilution, yielding phage that contains a high proportion of host-range mutants.

**Mutant types.** The mutants observed are identified by plating phage on agar seeded with a mixture of *E. coli* strain B (Delbrück) and one of its mutants, B/3b, resistant to T<sub>3</sub>. The plates are incubated at 38.5° for 3 hours and then allowed to stand at room temperature for several more hours, during which time they are observed at intervals and variant plaques are

marked. All particles form plaques, and variants are picked by inspection.

The phenotypes observed under these conditions may be roughly classified into six types (the wild type gives phenotype III): (I) uniformly turbid; (II) turbid with a slight ring of clearing at the periphery and occasional clear areas in the center; (III) turbid with a clearing ring at the periphery and irregular clear areas in the center of each plaque; (IV) turbid with a marked clearing ring and an irregular clear area in the center (these plaques soon become entirely clear, except for a fuzzy edge); (V) clear with a narrow halo; (VI) clear with a sharp edge. Each of these phenotypic classes includes phage strains differing in genetic constitution. The differences are heritable and, apart from the production of further mutants, do not depend on the bacterial strain in which the phages are grown.

**Distribution.** Single bursts produced by complexes of B and wild-type T<sub>3</sub> diluted after 4 hours in antiserum were studied. It was found that mutants occurred in bursts which usually also contained a majority of wild-type particles. There seemed to be a correlation between the frequencies of phenotype I and phenotype IV or V in individual bursts. Early lysates of complexes were made by the cyanide technique at 2-minute intervals after "induction" by dilution in broth at pH 6. Variants were picked from plates of each of these lysates and replated. Fifty per cent of the variant plaques from the earliest yield contained approximately equal mixtures of phenotypes I and IV or V. The proportion of mixed plaques decreased in later lysates, reaching 15 per cent at the time of normal lysis. Thus it appears that the variants must be formed in pairs by a single event. This is hard to explain by a theory of mutational origin of the variants.

**Irradiation of the host.** Ultraviolet irradiation of the bacteria before infection increases the frequency of mutation. In one experiment, B cells were irradiated for various lengths of time, infected with T<sub>3</sub>



wild type, and plated 10 minutes after infection. The viral yield from nonirradiated cells contained no variants among 4280 plaques. With increasing ultraviolet dose the proportion of mutants of all types increased, to reach a maximum of about 1 per cent in the yield of cells that had been irradiated 2 minutes. (One minute gave a bacterial survival of  $5 \times 10^{-6}$ .) In another experiment it was found that the mutants occurred in bursts containing a majority of wild-type particles. The mutants appeared to be the same phenotypes as those resulting from complex formation with B. This is in agreement with Jacob's results on the production of viral mutants by irradiation of the host cell (K-12S) before infection with nonirradiated phage ( $\lambda$ ). The mutants in such experiments may be produced by the action of mutagenic substances in the cytoplasm of the irradiated bacteria, or may reflect some more specific type of phage-bacterium interaction.

*Possible origin of mutants.* The initial observation, that mutants differing from wild type in several loci had presumably been formed by a single event, suggested that the event in question might be a recombination rather than a mutation.

If the mutants arise as a result of recombination between variants of identical phenotype already present in the wild-type stock, they should not be found in the progeny of singly infected complexes. This point was tested by single-burst experiments with complexes formed by single infection. Mutants occurred with the usual frequency in these bursts. Therefore, if they arise by a process of recombination, it cannot be by recombination between two different strains of phage in the wild-type stock.

During the past year, the idea has been discussed that there may be an actual homology between the genetic material of the phage and part of that of the host cell. To test this possibility, the interaction between B and T<sub>3</sub> has been provisionally regarded as a genetic cross, and on that

basis predictions have been set up to be tested experimentally.

If one of the parents in a cross is changed, the nature of the recombinants should be changed. Then hosts differing in the chromosome section homologous to the phage in question should produce different classes of mutant. A study was made of the mutant phenotypes formed by complexes of T<sub>3</sub> wild type and each of a number of B strains that had been carried independently for some years. With six such strains, the total number of mutants found did not differ significantly from strain to strain, but with three of them the ratio of the numbers of variants IV, V, and VI to I and II diverged widely from the value 0.56 found with the original B. These values ranged from 0.08 in strain S (Hershey) to 2.0 in strain B' (Hershey). The efficiency of plating of each phenotype on the various strains did not give any indication of selective differences that might account for the variations found. The possibility of intracellular selection should be investigated by means of further experiments.

In a genetic cross the recombinants are closer in genetic constitution to either parent than the two parents are to each other. If a recombinant is backcrossed to one of the original parents, the new recombinants will be closer still to that parent, and the process of isolation of successive recombinants of backcrosses may be continued, to approach the genetic constitution of the parent more and more closely. By a series of "backcrosses" of phage to bacterium—that is, by isolation of a series of successive mutants on the same bacterial strain—it might be possible to produce a phage strain which would not give rise to mutants because the homologous portions of the genetic material of phage and bacterium would be identical. This principle might permit a critical test of the hypothesis under consideration.

Preliminary work has been done along these lines. Three phage strains, nos. 3, 14, and 18, all giving no further mutants

on B, have been derived from wild type by successive isolation of mutants through the following steps (phenotypes shown in parentheses):

No. 1 (III, wild type)  $\xrightarrow{\times B}$  no. 2 (IV)  $\xrightarrow{\times B}$  no. 3 (I);

No. 1 (III)  $\xrightarrow{\times B}$  no. 11 (IV)  $\xrightarrow{\times B}$  no. 14 (I);

No. 1 (III)  $\xrightarrow{\times B}$  no. 4 (I)  $\xrightarrow{\times B}$  no. 7 (V)  $\xrightarrow{\times B}$  no. 10 (IV)  $\xrightarrow{\times B}$  no. 18 (I).

No. 3 and no. 14 appear to be identical, and produce no recombinants when crossed together by the standard phage-cross technique. The intermediates in the isolation series, no. 2 and no. 11, are not identical. No. 18 is clearly different from no. 3 and from no. 14.

The yields from complexes of these three strains on a different host strain, B', all include mutant types. The mutants produced by no. 3 and no. 14 on B' have identical phenotypes and appear in about equal numbers. No. 18 on B' gives mutants of another phenotype. Thus the stability of nos. 3, 14, and 18 is specific for the host in which they were obtained. In terms of the hypothesis, these strains, which give no recombinants when crossed with B, do give recombinants when crossed with B'.

*Relationship of the stable strains.* It was thought possible that strains no. 3 and no. 18 might not be completely homologous with respect to each other if they had picked up, from the host, genetic material not entirely homologous to T<sub>3</sub>, and different in each case. They were crossed together, and two recombinants were isolated. The most conspicuous recombinant, of phenotype VI, comprises about 12 per cent of the yield, a very high percentage for T<sub>3</sub>. The second recombinant gives a very small, ragged plaque of phenotype I. If no. 3 and no. 18 were not completely homologous, it would be expected that the recombinants might differ in length of chromosome (and hence in size) from each other and from each parent. High-speed centrifugations of mixtures of the

two recombinants, and of the clear recombinant (VI) with each parent, show that the clear recombinant sediments less rapidly than any of the other strains. The

techniques so far employed do not permit a definite conclusion as to whether this result is due to an actual difference in size of the phage particles, or to some fortuitous factor. A more convincing demonstration of a difference in amount of genetic material per phage particle might be obtained from studies of ultraviolet inactivation of mixtures. Work on this possibility is in progress.

#### PREASSIMILATED BACTERIAL SOURCES OF VIRAL MATERIAL

*Sources of DNA.* When C<sub>14</sub>-labeled bacteria are infected with T<sub>2</sub> in nonradioactive glucose medium, radioactivity rapidly disappears from the DNA cytosine and simultaneously appears in DNA hydroxymethyl cytosine (fig. 1). If, instead, unlabeled bacteria are infected in radioactive glucose, radioactivity appears in DNA hydroxymethyl cytosine but not in cytosine. As Kozloff found, and we have confirmed, bacteria specifically labeled in DNA thymine transfer their label to viral DNA. These results show that some of the materials used for synthesis of viral DNA come from preformed bacterial DNA, others come from the glucose assimilated after infection, and bacterial DNA is not an intermediate along the second route. This means that the infection alters DNA metabolism in three ways: it stops formation of bacterial DNA instantly, it quickly initiates the synthesis of viral DNA, and it causes the existing bacterial DNA to all but disappear.

These facts suggest that the rapid con-



version of bacterial DNA into viral DNA is probably quite as unique as the accompanying genetic events certainly are. This line of thought led us to inquire whether the virus initiates the breakdown of bacterial DNA, or merely intercepts the products of a normal breakdown mechanism, and whether bacterial DNA is the only characteristic bacterial substance used to make virus.

It turns out that bacterial DNA is not the only source of preassimilated materials used to make viral DNA. If, in the experi-

ment in which one observes the entry of preassimilated  $C_{14}$  into viral DNA, one looks at any of the purines and pyrimidines other than the cytosines, one observes a slow, continuous increase in radioactivity. This is shown for thymine in figure 2. The rise usually amounts to 30 per cent or more 90 minutes after infection, and is not an analytical artifact because it does not occur when the cells have been specifically labeled by feeding radioactive thymidine.

Table 3 shows the results. (Radioactivi-

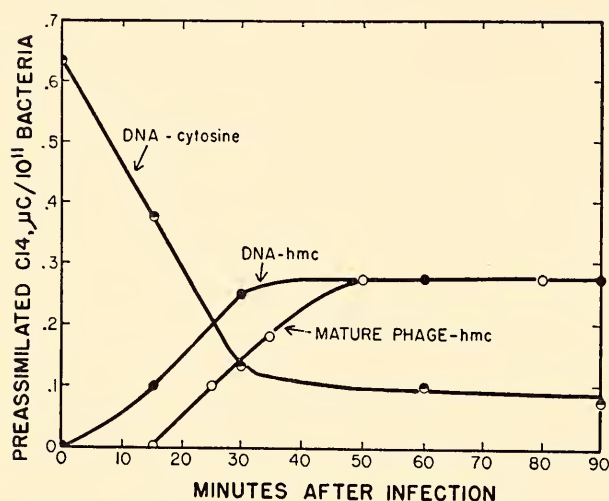


FIG. 1. Destruction of bacterial DNA and formation of viral DNA as observed by analysis of preassimilated  $C_{14}$  in cytosine and 5-hydroxymethyl cytosine (hmc) after infection.

ment in which one observes the entry of preassimilated  $C_{14}$  into viral DNA, one looks at any of the purines and pyrimidines other than the cytosines, one observes a slow, continuous increase in radioactivity. This is shown for thymine in figure 2. The rise usually amounts to 30 per cent or more 90 minutes after infection, and is not an analytical artifact because it does not occur when the cells have been specifically labeled by feeding radioactive thymidine.

The following experiment shows that the previously undetected source is probably ribose nucleic acid (RNA). Bacteria were labeled differentially by growing them in  $C_{14}$  glucose supplemented with nonradioactive uridine, cytosine, and orotic

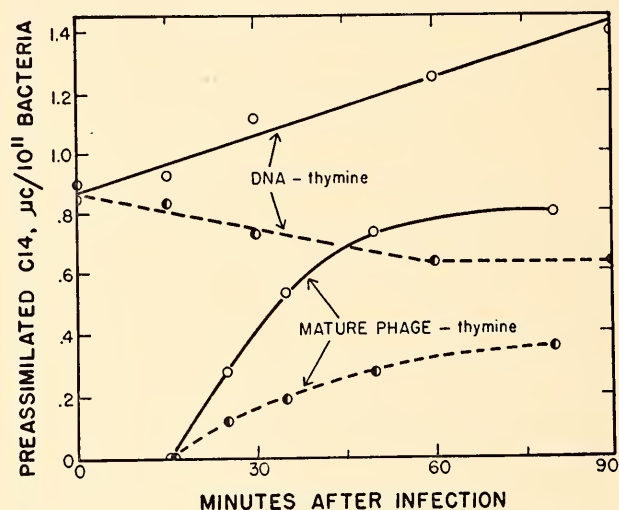


FIG. 2. Conversion of preassimilated  $C_{14}$  into DNA thymine and into phage thymine in infected bacteria. Continuous lines, culture without competitor; dashed lines, parallel culture with added thymidine. Phage growth was linear after 15 minutes, reaching 150 to 180 per bacterium at 80 minutes in both cultures.

ties shown for the pyrimidines have been corrected by subtracting assays of blanks cut from the rims of the spots, and are not very precise.) It can be seen that purine radioactivity increased significantly after infection, but no radioactivity entered the DNA pyrimidines. This result shows that the rise in pyrimidine radioactivity observed in similar experiments with uniformly labeled cells (fig. 2) comes from RNA or from some unknown source of preformed, metabolically stable pyrimidines.

The likelihood that the source is RNA is supported by the observation that labeled

RNA phosphorus and carbon decrease after infection, more than enough to account for the rise in labeled DNA. This decrease is slow and small, however, never exceeding 10 to 20 per cent of the total RNA, and does not lessen the special significance we wish to attach to the dramatic conversion of bacterial DNA into viral DNA illustrated in figure 1.

TABLE 3

DISTRIBUTION OF DNA C14 BEFORE AND AFTER INFECTION IN BACTERIA LABELED BY GROWTH IN C14 GLUCOSE (0.025  $\mu\text{C}/\mu\text{g}$  C UNIFORM LABEL) PLUS NONRADIOACTIVE URIDINE

| TIME<br>AFTER<br>INFECTION | $\mu\text{C}$ C14 PER $10^{13}$ BACTERIA |     |               |              |              |
|----------------------------|--|-----|---------------|--------------|--------------|
|                            | Gua-<br>nine                             | Hmc | Cyto-<br>sine | Ade-<br>nine | Thy-<br>mine |
| 0 minutes                  | 57                                       | 0   | 0             | 61           | 10           |
| 90 minutes                 | 75                                       | 0   | 0             | 79           | 8            |

*Sources of protein.* The following experiments were designed to measure the extent to which preassimilated bacterial sulfur is used to make viral protein in infected bacteria.

Bacteria were labeled with radioactive sulfur ( $\text{S}^{35}$ ), washed free from external  $\text{S}^{35}$ , and allowed to grow for 30 minutes in nonradioactive medium (glucose, sulfate, phosphate, tris hydroxymethylamino methane buffer, salts). Such cells contain radioactive proteins and also relatively large amounts of labeled glutathione, which is a potential protein precursor but is not used up except in cells starved for sulfur (Abelson *et al.*, Year Book No. 52, pp. 133-135).

Such labeled cells were infected with  $\text{T}_2$ , and samples were lysed in the presence of cyanide and a large excess of lysing phage (inactivated by ultraviolet light) at various times during the period of viral growth. The lysed samples were treated with ribonuclease and deoxyribonuclease before fractionation, which reduced the radioactivity of the small-particle fraction from uninfected bacteria very appreciably.

Carrier bacteria (heat-killed B/2), which do not adsorb the phage, were then added, and the lysates were separated into a large-particle fraction and a small-particle fraction by successive low- and high-speed centrifugation. Other details of these methods are given in previous publications from this laboratory.  $\text{S}^{35}$  was assayed in the sedimentable fractions of the lysates, and also in trichloroacetic acid precipitates ("total protein") of other samples with-

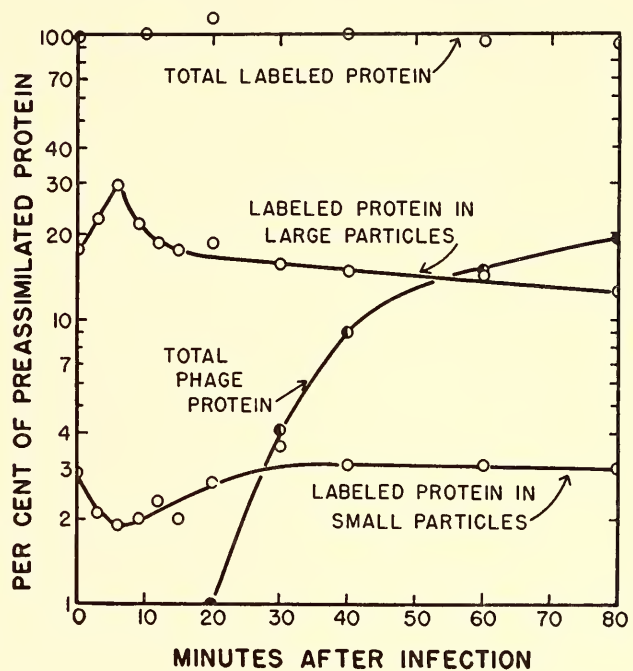


FIG. 3. Distribution of protein labeled with preassimilated  $\text{S}^{35}$  after lysis of infected cells. Preassimilated protein sulfur per bacterium measured 1060 phage equivalents ( $2.8 \times 10^{-9}$   $\mu\text{g}$ ). Total phage protein is computed from the yields of infective particles, for example, 200 per bacterium at 80 minutes.

drawn from the infected culture at various times. Yields of phage were measured by plaque titrations of the lysates; 90 per cent of the infective particles were recovered in the small-particle fraction.

Figure 3 shows the results of a typical experiment of this kind. The data are expressed as percentages of the total labeled protein assimilated before infection. These can be converted into micrograms of protein sulfur per bacterium from conversion factors (given in the legend) computed from the specific activity of the preassimi-



lated sulfur and corrected for the 30-minute period of growth in nonradioactive medium before infection. Total sulfur in phage protein is computed from the results of plaque titrations, multiplied by the sulfur content per phage particle ( $2.5 \times 10^{-12}$   $\mu$ g). The two basic constants, protein sulfur per bacterium and per phage, have been measured radiochemically in numerous independent experiments, some of which are described in a later part of this report.

The main facts shown in figure 3 are as follows. First, total labeled protein remains constant after infection. Second, labeled protein in small particles does not show a significant rise correlated with the formation of mature phage particles. Third, the initial fall and subsequent rise in S<sub>35</sub> content of the small particles is inversely correlated with changes in the large particles. This is the effect of a temporary resistance to lysis characteristic of infected bacteria, first described by Visconti and also noted visually in the present experiments.

These facts show that there is no extensive conversion of preassimilated sulfur into phage protein. On the other hand, it is clear that centrifugal fractionation leaves about 3 per cent of the total bacterial protein, equivalent to 32 phage particles per bacterium or 16 per cent of the phage yield at 80 minutes, as a contaminant of the small-particle fraction. More specific methods of purification are required to detect smaller amounts of labeled sulfur that may be contained in the phage particles.

Adsorption to bacteria offers one such method. Two phage preparations, both isolated from the culture described in figure 3, were examined by this means. One consisted of the small-particle fraction obtained after lysis at 60 minutes, and contained 151 phage particles per bacterium. The other was obtained after spontaneous lysis yielding 500 phage per bacterium, and was purified by repeated fractional centrifugation, during which only 27 per cent of the phage was recovered.

Samples of phage were mixed with suffi-

cient numbers of heat-killed bacteria (strain H) to adsorb all the phage (98 per cent by titration), and measurements were made of radioactivity sedimentable with the bacteria or precipitable from their supernatant fluid by trichloroacetic acid. The specificity of adsorption was controlled by identical measurements using similar numbers of heat-killed bacteria (strain B/2) that did not adsorb the phage. The sensitivity of the measurement was greatly increased by a preliminary treatment of each sample with B/2, which served to remove material adsorbed or sedimented nonspecifically. The assay methods were controlled by requiring that the radioactivity of sediment and supernatant fractions account for the total input (all measured under conditions of negligible self-absorption). Results are given in table 4.

The table shows, as concluded previously, that most of the radioactivity in the material from the 60-minute lysate is contained in contaminating bacterial protein. Only 12 per cent of the radioactivity, corresponding to 0.4 per cent of the labeled bacterial protein, or 2.6 per cent of the total phage protein, fails to be separated from the phage by this method. Of the radioactivity contained in the phage isolated from the terminal lysate, about half is specifically adsorbable. This half, after correction for the loss of phage during purification, corresponds to 1.1 per cent of the total labeled bacterial protein, or 2.2 per cent of the terminal yield of phage protein.

We conclude that preassimilated sources of phage sulfur, like preassimilated sources of phage DNA other than bacterial DNA, are drawn on slowly and inefficiently as compared with the utilization of the bacterial DNA itself.

These experiments do not prove that any bacterial protein is converted into phage protein. To test this, one would have to use labeled bacteria that had been starved to deplete their acid-soluble reserves (Abelson *et al.*).

*Nucleic acid turnover in uninfected bac-*

*teria*. Bacterial DNA is destroyed quickly, and RNA slowly, in infected bacteria. Does the virus initiate these decompositions, or merely block resynthesis?

If bacterial RNA or DNA or both were continuously being broken down to the level of common precursors of these two materials, one would expect, except under very special conditions, that labeled purines and pyrimidines would pass preferentially in one direction between them, and perhaps out of both, during the growth of labeled cells in unlabeled medium. More certainly, the addition of specific unlabeled

We conclude that if any breakdown of nucleic acid occurs during bacterial growth, it involves only a very small percentage per generation of the bacterial DNA, and still less of the more abundant RNA, or it yields unknown intermediates not interceptable by the available competitors. It follows that the rapid breakdown of DNA in infected bacteria is stimulated by the infection.

It should be noted that our experiments do not exclude time rates of turnover comparable with those observed in the classic experiments with resting mammalian tis-

TABLE 4

TEST FOR CONSTITUENT S35 IN PHAGE GROWN ON S35-LABELED BACTERIA

(The sediments contain S35 adsorbed to successive additions of heat-killed bacteria; the supernatant contains residual unadsorbed S35.)

| FRACTION ANALYZED                     | RADIOACTIVITY (CPM PER ML) |      |                |      |
|---------------------------------------|----------------------------|------|----------------|------|
|                                       | 60-minute yield            |      | Terminal yield |      |
|                                       | (1)                        | (2)  | (1)            | (2)  |
| First sediment (B/2).....             | 1100                       | 1220 | 129            | 140  |
| Second sediment (B/2).....            | 330                        | ...  | 114            | ...  |
| Second sediment (B).....              | ...                        | 659  | ...            | 785  |
| Second supernatant .....              | 1045                       | 825  | 915            | 320  |
| Whole sample .....                    | ...                        | 2750 | ...            | 1420 |
| Percentage specifically adsorbed..... | ...                        | 12   | ...            | 50   |

precursors to the medium would cause specific losses of labeled constituents in a manner predictable from the results of ordinary isotope-competition experiments.

Breakdown of nucleic acids in uninfected bacteria was tested along the lines indicated, using cells uniformly labeled by feeding radioactive glucose, and allowing these to grow for 7 generations (6 hours) in the presence of nonradioactive glucose supplemented with ribo- or deoxyribonucleosides. At the end of this period of growth, the bacterial DNA was analyzed for labeled purines and pyrimidines. No difference in amount or distribution of radioactivity was found between the DNA in the terminal and that in the starting cultures, for any of five competitors tested.

sues, in which the period of observation generally spanned several days. The point of our findings, which are in agreement with other results for microorganisms, is that rapid synthesis of cell constituents does not call for an acceleration of the slow processes of decay that could doubtless be observed also in microbial cultures by experiments of sufficient duration.

*Biochemical route from bacterial to viral DNA.* As already shown, the intermediates in the transfer of labeled viral DNA from parents to offspring cannot be intercepted by supplementing the culture medium with specific DNA precursors. Before concluding from this that the transfer is, in some sense, direct, it is necessary to study by the same methods a comparable



situation in which the transfer is indirect. The transfer from bacterial DNA to virus recommended itself for the purpose. This simple aim has broadened considerably in the course of the work, leading us and our collaborators in Toronto to undertake some rather ambitious experiments. The present stage of the project can be summarized only briefly here.

When nonradioactive purines or pyrimidines, or their nucleosides or nucleotides, are put in competition with the conversion of labeled bacterial DNA to viral DNA, a decided but incomplete suppression of the transfer of thymine is observed in response to thymidine. This effect is illustrated in figure 2. Thymidylic acid also has a slight effect, and other competitors seem to have specific effects, but these are so small that it is difficult to be sure of them.

The competition observed with thymidine operates equally well when the bacterial DNA thymine is specifically labeled, which shows that the observed competition is not directed primarily against non-DNA sources.

These results partly confirm our expectations, but serve chiefly to raise new questions. On the one hand, we find that competition between bacterial DNA and external substrates as precursors of viral DNA can be demonstrated, and wish to contrast this evidence of indirect use with the failure of competition in the use of the parental viral DNA. On the other hand, the observed competition is severely limited, showing that the use of bacterial DNA by the virus does not involve primarily simple intermediates such as free bases, nucleosides, and perhaps nucleotides. This we wish to interpret as an indication of close biological and biochemical relationships between the two kinds of DNA, in spite of their obvious differences. Whether this confusion of ideas will lead any farther remains to be seen.

Both the biochemical and the general interpretation of these results depend on experiments in progress to locate the point

of competition by thymidine, presumably some late step in the synthesis of DNA. It may be that this system will prove to be uniquely suited to such questions, the answers to which are essential for either genetical or biochemical thinking about DNA.

#### SYNTHESIS OF VIRAL PROTEIN

Our immediate aim in studying protein synthesis in infected bacteria is to answer the question: Is protein synthesis part of, or otherwise necessary for, the replication of genetic determinants? As a start we have examined the assimilation of radioactive sulfur, and its incorporation into protein and virus, at various times after infection. The preliminary results already permit some interesting deductions.

As mentioned previously, bacterial protein formed before infection is not available in appreciable amounts for conversion into viral protein. This allows us to focus on proteins formed after infection.

If S35 is added to a bacterial culture immediately after infection, its incorporation into acid-insoluble materials occurs at nearly the rate characteristic of uninfected bacteria. This was shown by adding S35 to a growing bacterial culture, immediately dividing it into two parts, and infecting one part with T2. Labeled sulfur subsequently incorporated into acid-insoluble materials was then measured at intervals in both cultures. Results will be expressed here in multiples of the sulfur content of one phage particle ( $2.5 \times 10^{-12}$   $\mu$ g). In the uninfected culture, labeled sulfur was assimilated at the initial rate of 10.6 phage equivalents per bacterium per minute, and measured 820 equivalents at the end of one generation (51 minutes). In the infected culture, the rate was initially 8.3 phage equivalents per bacterium per minute, and gradually decreased after 30 minutes. In another experiment the rate for the infected culture was 90 per cent of that for the uninfected. The difference, and especially the decreasing rate after 30 minutes, must be due in part to early lysis of

some of the cells. We conclude, in confirmation of Cohen's early experiments, that protein synthesis continues without interruption during and after infection.

Figure 4 shows an infected culture analyzed more completely by this method. The acid-insoluble sulfur content per bacterium at the time of infection (measured radiochemically from a parallel culture uniformly labeled with S35) was 1040 phage equivalents. At the observed growth rate of 1.18 generations per hour, this must have been formed at the exponential rate

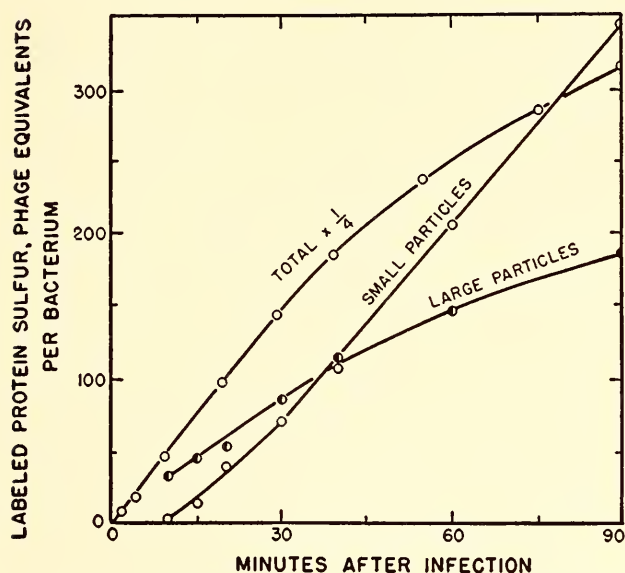


FIG. 4. Incorporation of S35 assimilated after infection into acid-insoluble protein and particulate fractions of lysates. S35 added at time zero. Multiply scale by 4 to read total assimilation.

of 1.35 per cent per minute. Converted to the equivalent linear rate for nonmultiplying cells, this amounts to 14 phage equivalents per bacterium per minute. The observed assimilation was linear during the first 30 minutes, and measured 19 equivalents per bacterium per minute, the excessive rate being due in part to the fact that an appreciable fraction of the sulfate in the medium had already been assimilated before the start of the experiment.

How much of this protein is a precursor of viral protein? Figure 4 shows that much of it is not, especially that portion that is incorporated into large particles.

Moreover, at 30 minutes after infection, labeled protein is being formed at the rate of 19 equivalents per bacterium per minute, whereas labeled protein in small particles is being formed at a rate of 4.8 equivalents per bacterium per minute, showing that only 25 per cent of the total protein being synthesized is going into phage.

Figure 5 shows the relation between the phage titer of lysates prepared at successive times and the labeled protein content of the small-particle fractions isolated from

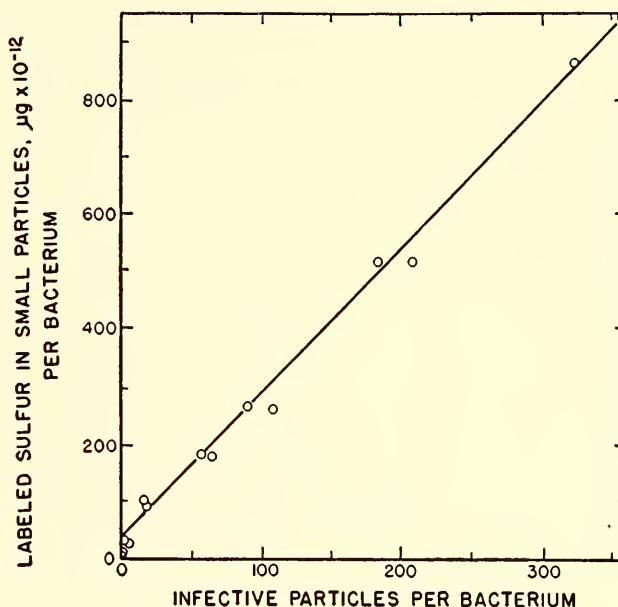


FIG. 5. Relation between phage titer and post-assimilated S35 content of small particles in lysates prepared at successive times after infection.

them, for the experiment just described. Such data justify the conclusion that most of the labeled protein is in phage particles, and this was, in fact, confirmed by tests of adsorption to bacteria. The slope of the straight line in figure 5 corresponds to  $2.5 \times 10^{-12}$  μg of sulfur per phage, in good agreement with other radiochemical analyses.

We have shown that the infected bacterium synthesizes considerably more protein than is incorporated into phage particles. From figure 4 it can be seen that the rate of synthesis of phage protein increases in relation to the rate of total protein synthesis as the infection progresses, an effect suggesting that the cells gradually



change over from one kind of protein synthesis to another. The following type of experiment was designed to analyze this phenomenon in greater detail. It also permits an estimate of the amount of viral-precursor protein per infected bacterium.

S<sub>35</sub> was added to infected cultures at various times after infection, and the assimilation was stopped 4 or 5 minutes later by adding sufficient neutral ammonium sulfate to reduce the specific activity of the medium 200-fold. The subsequent incorporation of S<sub>35</sub> into protein and into phage

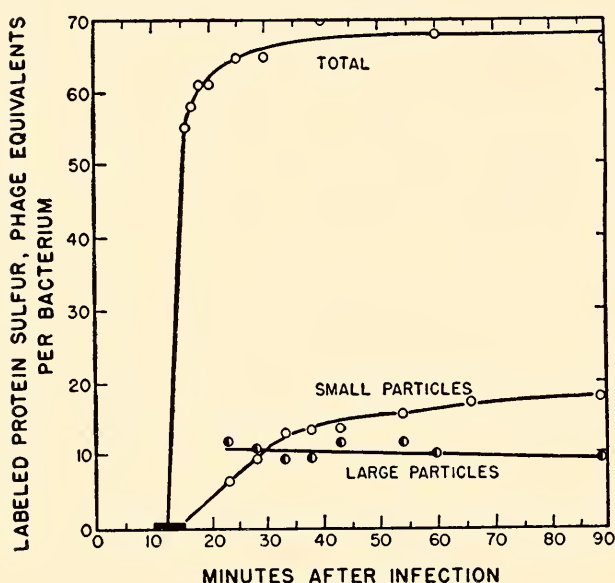


FIG. 6. Incorporation of S<sub>35</sub> assimilated between 10 and 15 minutes after infection into acid-insoluble protein and particulate fractions of lysates. Phage growth was linear after 20 minutes, reaching 125 per bacterium at 89 minutes.

particles was then assayed periodically, as already described.

Figure 6 shows one of a series of experiments of this type that are consistent among themselves, in which the first virus particles begin to form at 20 minutes instead of the usual 15. (The slow phage growth was eventually traced to a deficiency of iron in the culture medium.) In this experiment, S<sub>35</sub> was assimilated between 10 and 15 minutes after infection. The principal results may be summarized as follows. (1) The total amount of labeled protein synthesized corresponds to 13.6 phage equivalents of sulfur assimilated

per bacterium per minute during the 5-minute period, in close agreement with the rate of synthesis by uninfected bacteria. (2) Most of the labeled protein is formed during the period of assimilation of S<sub>35</sub>, and the maximum amount is reached within a few minutes afterward. The rate of incorporation is conveniently measured in terms of the interval between the midpoint of the assimilation period and the time when half the maximum labeled protein has been formed—about 2 minutes. (3) The corresponding time for the half-maximum rise in labeled phage protein is 13 minutes. If we assume that this protein was formed with the 2-minute half-time of the total protein, it must have spent about 11 minutes in the cell as phage-precursor protein. (4) Phage-precursor protein receiving its label from the sulfur assimilated between 10 and 15 minutes measures 22 per cent of the total protein similarly labeled.

Other experiments of this series showed that only about 5 per cent of the protein labeled by assimilation between 1 and 5 minutes after infection was phage-precursor protein, and that the proportion increased to 50 or 60 per cent for assimilation between 30 and 35 minutes. Atoms assimilated late persisted in phage-precursor protein for about 8 minutes. Similar experiments in which viral-precursor DNA was labeled with P<sub>32</sub> under the same conditions showed that the time spent in DNA precursor was about 14 minutes. These results seem to show that the pool of viral-precursor DNA is nearly twice as large (in terms of phage equivalents) as the pool of viral-precursor protein, and therefore that the DNA that enters a given virus particle is formed earlier, on the average, than the bulk of its protein. The precision of our experiments leaves something to be desired, however, and we propose to seek confirmation of this conclusion by other methods.

The efficiency of conversion of labeled protein into phage as a function of the time of assimilation of S<sub>35</sub> has been meas-

ured with satisfactory accuracy. Table 5 presents the results of a series of simultaneous measurements, made by the method just described except that total labeled protein was measured by a single analysis 30 minutes after the beginning of the interval

TABLE 5

RATE OF INCORPORATION OF S35 INTO TOTAL PROTEIN AND PHAGE PRECURSOR AT VARIOUS TIMES AFTER INFECTION

(S/B/min = phage equivalents of sulfur assimilated per bacterium per minute)

| Interval tested (min)      | Total protein (S/B/min) | Phage precursor (S/B/min) |
|----------------------------|-------------------------|---------------------------|
| Before infection . . . . . | 13.2                    | ..                        |
| 1-5 . . . . .              | 14.5                    | 1.9                       |
| 5-10 . . . . .             | 12.4                    | 3.1                       |
| 10-15 . . . . .            | 9.4                     | 5.0                       |
| 16-21 . . . . .            | 9.0                     | 5.3                       |
| 22-27 . . . . .            | 8.2                     | 4.6                       |

tested, and labeled phage protein by analysis after an additional 30 minutes. The culture medium was supplemented with  $10^{-5}$  M  $\text{FeCl}_3$ . As was found before, the efficiency rises to a maximum of 53 to 59 per cent as the infection progresses, al-

though the time scale is altered by the more rapid growth of phage. The limit is not imposed by early lysis of cells, because a control culture that assimilated P32 between 4 and 8 minutes after infection showed an efficiency of incorporation of labeled DNA into phage of 76 per cent.

In summary, we have preliminary evidence that the synthesis of viral DNA precedes the synthesis of viral protein, and very good evidence that infected cells form at least two classes of protein, one viral-precursor and one not. During the course of the infection the ratio of precursor to total protein being synthesized gradually increases, but never exceeds about 60 per cent. Whether or not the nonprecursor protein is virus specific in some other sense, and what role it may play in viral growth, our experiments do not tell. The fact that no sudden change in rate of synthesis follows infection, however, suggests that the infected cell continues to make most of the proteins it was making before. If so, the failure of infected cells to form typical bacterial enzymes must be regarded as an exceptional circumstance that itself deserves further investigation.

## BACTERIAL GENETICS—I

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Until last fall we had used strain B of *Escherichia coli* exclusively in our studies of spontaneous and induced mutability. A serious drawback was that methods were not available in this material for making standard genetic analyses, such as determinations of whether or not similar mutants are allelic. Recently, methods have been developed for crossing strains B and K-12 of *E. coli*, and these should be very helpful in our studies. Before we were aware of this new development, however, we started work with *Salmonella typhimurium*, because Zinder and Lederberg had shown that transduction can be used to determine allelic relationships, and be-

cause from the technical standpoint *Salmonella* is very suitable for our studies.

Thus during the past year we have been experimenting with both *E. coli* and *S. typhimurium*. Using *E. coli*, we have continued comparative studies of the action of mutagens on individual genes, have investigated certain factors that modify the mutagenic effectiveness of ultraviolet radiation, have studied spontaneous mutability in growing bacteria with the aid of the chemostat, and have tested for mutagenicity a number of compounds which are of interest in connection with cancer chemotherapy. Using *Salmonella*, we have studied allelism and pseudoallelism in a large



number of auxotrophs, have observed the action of certain mutagens on different alleles of the same locus, and have investigated problems related to the mechanism of transduction.

In addition to the workers named above, our group included Mrs. G. C. Smith, who had charge of the *Drosophila* colony, and Mrs. Katherine M. Main and Mrs. Jean W. McIntyre, who washed and sterilized the glassware and prepared the culture media used in the bacterial experiments. During the summer of 1954 Constance and David L. Bryson helped with routine work.

As guest investigators during the summer of 1954 our group included Dr. Joseph S. Gots of the Department of Microbiology of the University of Pennsylvania, and Dr. Sydney Brenner of the Department of Physiology of the University of the Witwatersrand, Johannesburg, South Africa, who was in the United States as a Fellow of the Carnegie Corporation of New York. In July 1954, Dr. Philip E. Hartman came to work at this laboratory as a Research Fellow of the Public Health Service.

Our work received partial support from a grant-in-aid from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council. A grant from the American Cancer Society also supported the tests of various chemicals for mutagenicity, which were carried out here as part of a Chemotherapy Screening Program organized by Dr. Alfred Gellhorn of the College of Physicians and Surgeons of Columbia University.

#### ANALYSIS OF SIMILAR AUXOTROPHS

When we began work with *Salmonella* our plan was to repeat with that organism the experiments we had already carried out with *E. coli*, in order to compare the action of various mutagens, using auxotrophs deficient in amino acid synthesis. In addition, we planned to extend that study to include auxotrophs deficient in the synthesis of purines and pyrimidines and the fermentation of sugars, and to use

transduction as a means of determining which of a series of mutants showing the same property were allelic and which were due to mutations occurring at different loci. As the first step in our work, we obtained a large number of independently originating auxotrophs. Our collection now includes 40 strains deficient for cystine, 27 deficient for histidine, 11 for isoleucine, 11 for leucine, 15 for methionine, 22 for proline, 29 for serine, 11 for tryptophane, 13 for adenine, and 12 for adenine plus thiamine, as well as 46 strains that are unable to ferment galactose. Next, we proceeded with tests for allelism by preparing cultures of temperate phages and using them in transduction experiments. It has been shown by Zinder and Lederberg (1952) that phage raised on wild-type bacteria is able to induce changes to wild type in a small fraction (one per  $10^5$  to  $10^3$ ) of mutant bacteria, but that phage raised on mutant bacteria cannot transduce another population of mutant bacteria if the mutants are allelic. An extensive analysis of the transduction phenomenon suggests, as the most probable mechanism, that a phage particle picks up a small segment of a chromosome belonging to the bacterium in which it originates, and, when it infects another bacterium, deposits this segment within it. Presumably, the segment then synapses with the homologous section of the chromosome of the host bacterium and during the divisions of that bacterium is in some way incorporated into the chromosome of one of its progeny.

*Cystineless types.* Transduction tests involving cystineless mutants, carried out by Blomstrand, showed that they can be divided into four groups according to the frequency of transductions observed when bacteria of one type are transduced with phage raised on bacteria belonging to another type. In tests between members of the same group, the number of transductions is considerably smaller than in experiments involving members of different groups or using phage raised on wild-type bacteria; and occasionally no transductions

at all are observed. Seven of the cystineless types belong to group A, 11 to group B, 8 to group C, and 5 to group D. Tests indicate that the cystine deficiency, in members of group A, can be partially satisfied by cystathionine, and in members of group C by either methionine, homocysteine, or cystathionine.

*Tryptophaneless types.* Ten tryptophaneless types were placed, as a result of transduction tests carried out by Nielsen, in four groups, designated A, B, C, and D. An analysis made by Brenner showed that the deficiencies of these four groups of auxotrophs involve four different reactions in the chain of synthesis of tryptophane, a situation identical with his previous findings in studies made with mutants of *E. coli*. The one mutant in group A fails to synthesize anthranilic acid; the group B mutants are unable to convert anthranilic acid to an as yet unidentified intermediate, called "compound B"; the group C type fails to convert compound B to indole; and those of group D are blocked in the conversion of indole to tryptophane.

*Histidineless types.* Experiments conducted by Hartman showed that 27 histidineless mutants fall, according to transduction tests, into seven groups (A to G), which coincide with groups obtained in biochemical studies of blocks in the synthesis of histidine. Groups D through G accumulate imidazoles, one of which (that accumulated by group G) appears to be histidinol and feeds derivatives of all the other groups. Groups B and C do not accumulate imidazoles, but, instead, a ninhydrin-positive substance which can readily be distinguished by chromatography. These two groups have not yet been separately characterized on the basis of accumulations, but a derivative of group B has been obtained which will feed on supernatants from group C cultures.

*Adenineless types.* According to transduction tests made by Yura, 12 adenineless types fall into five groups (A to E). Biochemical analysis by Gots showed that the nutritional requirement of all three mem-

bers of groups A and B can be satisfied only by adenine. All eight members of groups C and D, however, are able to grow on any one of four purines—adenine, hypoxanthine, guanine, and xanthine—and accumulate a yellow pigment which has a specific absorption at 3050 Å. This pigment is associated with a pentose and a diazotizable amine. One mutant, the only member of group E, grows on any one of four purines, but the purine requirement can also be supplied by either 4-amino-5-imidazolecarboxamide or glycine, or, less efficiently, by histidine. This mutant does not produce a yellow pigment, but accumulates a substance which has a specific absorption with peaks at 2450 and 2800 Å. The grouping arrived at by biochemical analysis thus coincides with the transduction-test grouping, except for the fact that biochemical tests have not yet been able to differentiate between some of the groups established by transduction.

*Galactose-negative types.* Forty-six independently originating galactose-negative mutants have been isolated by Zlata Demerec. Nine of these occurred in *Salmonella* strain LT2, and 37 in strain LT7. They could be separated into eight classes (I to VIII) on the basis of three criteria: appearance of colonies on EMB-plus-galactose medium; amount of residual growth on synthetic medium with galactose as a carbon source; and degree of instability, as shown by the formation of sectors or papillae on colonies grown on EMB medium, and by frequency of reversions to wild type on minimal medium.

I. Twenty of the mutants are included in the first group. When the bacteria are plated so as to form a film on EMB medium, the appearance of the growth is light purple. Single colonies are pink, darkening to purple on older plates. Residual growth on minimal medium is heavy.

II. The eight mutants in this class form a purple film on EMB medium. Colonies are purple, and smaller than those of class I. These mutants are unstable, producing light-pink papillae. Many reversions occur



on minimal medium. The amount of residual growth is fair.

III. Five mutants were found which form a dark-purple film on EMB medium, developing a sheen on older plates. Single colonies are dark purple and mucoid. Instability is expressed by the formation of wild-type papillae on older colonies. Residual growth on minimal medium is light. These mutants are extremely unstable, having a high reversion rate on minimal medium.

IV. One mutant is so highly unstable, mutating to wild type with such a high frequency, that the film on EMB appears to have a metallic sheen. Single colonies are purple, but show wild-type papillae and sectors. On minimal medium this mutant has a high reversion rate.

V. One mutant produces a dark-purple film on EMB. The colonies are dark purple and unstable, forming many wild-type sectors and papillae as well as pink papillae. The residual growth on minimal medium is very heavy.

VI. In eight of the mutants the film on EMB medium shows a metallic sheen. Single colonies have this sheen only in spots. Mutability could not be studied in these eight mutants, because of their very heavy residual growth on minimal medium.

VII. Two of the mutants produce a purple film on EMB, but a metallic sheen appears in the surrounding medium. Single colonies are rough purple with a slight sheen. These mutants are stable; no sectors or papillae appear. On minimal medium the residual growth is very heavy.

VIII. This mutant also forms a dark-purple film on EMB; single colonies are dark purple with a slight sheen. It is highly unstable, as many papillae appear on older plates. The residual growth on minimal medium is very heavy.

In class II, consisting of 8 types, and in the 5 types of class III, residual growth is sufficiently limited so that it is possible to detect both transductions and reverse mutations. Transduction tests made with bac-

teria from class II and phage raised on bacteria of class III produced a number of transductions as large as or larger than that obtained in experiments using phage raised on wild-type bacteria. This indicates that members of class II are not allelic to members of class III. In experiments with the 8 members of class II, no transduction occurred between *gal-17* and *gal-24*, and other combinations between members of the group showed numbers of transductions considerably smaller than when wild-type phage was used. Thus all the members of class II form one transduction group. Transduction experiments could not be carried out among the members of class III, because it appears that they are sensitive to temperate phage.

Studies of reverse mutation to wild type were made with the 8 types of class II and 3 of the types belonging to class III. All of them mutate spontaneously. The spontaneous-mutation rate of the class III types is so high that it was not feasible to use them in studies of induced mutability. Four mutagens were used in the experiment, namely, ultraviolet radiation, manganous chloride, triethyleneimino triazine, and diepoxybutane. One of the class II types was found to be mutagen stable, whereas in the other 7, mutations could not be induced by treatment with  $MnCl_2$  but were induced by treatment with any of the other three mutagens.

*Serineless types.* Transduction experiments and tests for induction of mutations were carried out by Zlata Demerec with 22 serineless types, which originated independently of one another. In all these the nutritional requirement can also be satisfied by glycine. Transduction tests revealed two groups, one (*serA*) comprising 21 types and the other (*serB*) one type only. Sixteen types belonging to group *serA* were treated with  $MnCl_2$ , ultraviolet rays, triazine, and diepoxybutane, which are the most potent of the mutagens we work with. The experiments with  $MnCl_2$  showed that this mutagen is much less potent for *Salmonella* than for *E. coli*. Of the 16

types tested, 7 were mutagen stable, that is, it was not possible to increase their spontaneous mutability by treatment with any of these mutagens. This high proportion of mutagen-stable types is an unusual feature of the results. In our work with *E. coli* we found about 18 per cent mutagen-stable types among the 40 studied, whereas in this case almost 44 per cent were mutagen stable.

*Prolineless types.* Experiments by Nielsen revealed two transduction-test groups, group A consisting of 13, and group B of 2 mutants.

#### PSEUDOALLELISM

The results just described show that similar auxotrophs can be separated by transduction tests into well defined groups. Between members of the same group, transduction either does not take place or is significantly less frequent than between these members and auxotrophs belonging to another group, or wild-type bacteria. The results also show that the grouping based on transduction tests coincides with that obtained by biochemical methods which investigate blocks in the chain of reactions leading to the synthesis of the compounds required by the auxotrophs. These results favor the assumption that members of each such group are allelic to one another, and that the occurrence of a small amount of transduction within a group can be explained on much the same basis as the infrequent recombination that takes place between pseudoalleles. They suggest that a gene locus extends over a section of a chromosome, and that changes occurring in different regions of this section give rise to different alleles. They also indicate that regions within a section may separate, and recombine—by a process analogous to crossing over—with homologous regions within a locus of another chromosome.

#### LINKAGE

As part of an effort to elucidate the mechanism of transduction, we have been

trying to find out if a transducing chromosomal segment might be of such a length as to include several adjacent loci. In this case two loci together would be altered by transduction, that is, they would show linkage. In order to test this, we developed strains with two or more mutant markers, by selecting additional mutations in strains that already had at least one. In eight such strains more than fifty new mutations, of spontaneous origin, were obtained. Transduction tests with these revealed one linkage group involving three loci: *tryA*, *cysB*, and *tryD*. We know of one mutant allele at the *tryA* locus (*tryA-8*), 11 at the *cysB* locus (*cysB-10*, *-12*, *-14*, *-15*, *-16*, *-18*, *-24*, *-25*, *-27*, *-40*, and *-41*), and 6 at the *tryD* locus (*tryD-1*, *-6*, *-7*, *-9*, *-10*, and *-11*). One experiment in which *tryA-8 cysB-12* bacteria were transduced by wild-type phage (that is, phage raised on wild-type bacteria) produced three phenotypic classes: 411 wild type, 625 *cysB-12*, and 958 *tryA-8*. Evidently the wild-type class originates when the piece of chromosome brought in by the transducing phage particle replaces the chromosome segment of the recipient bacterium which involves both the *tryA-8* and *cysB-12* loci; class *cysB-12* is obtained when the *tryA-8* locus is replaced, and class *tryA-8* when the *cysB-12* locus is replaced. From the experimental data it is evident that the frequency with which these two loci are replaced together is lower than the frequency with which either of them alone is replaced. Similar results were obtained in experiments with bacteria carrying *cysB-12* and *tryD-10*.

At present we have three bacterial stocks whose genotypes combine two of the three linked loci (*tryA-8 cysB-12*; *cysB-12 tryD-10*; *cysB-18 tryD-11*), as well as stocks in which all the alleles of these loci are represented singly. We also have a collection of phages raised on each of these bacterial stocks. This material is being used to study the genetic structure of loci, and to investigate the mechanism of transduction.



## MECHANISM OF TRANSDUCTION

On the basis of the results obtained by Zinder and Lederberg, and further work in our laboratory, transduction can be visualized as a process whereby a phage particle transfers a segment of chromosome from the bacterium in which it was raised to the bacterium which it infects. After infection this segment is in some way incorporated into the corresponding chromosome of the recipient bacterium, replacing a homologous region, and is so transmitted to the descendants of that bacterium. That the phage transfers only a segment of a chromosome is indicated by the failure of the above-mentioned workers to find transduction affecting two genes simultaneously, as would be expected if a whole chromosome were transferred.

The results of our experiments contribute further toward elucidation of the question of the size of the transferred segment. As was mentioned earlier, we have tested more than fifty double mutants for the occurrence of simultaneous transductions, and have found only one linkage group involving three loci. This indicates either that the segment must be fairly short, or—which is unlikely—that the number of chromosomes in *Salmonella* is considerably larger than has been supposed.

All available data are consistent with the assumption that incorporation of the transducing segment into the chromosome of the recipient bacterium occurs during chromosomal duplication. Presumably the segment synapses with the homologous region of the bacterial chromosome. Incorporation might be accomplished in one of two ways. (1) In the process of duplication every component of the bacterial chromosome may attract from among the materials present in the cell replicas of itself, which serve as building blocks for a new chromosome. When they join together to form it, the segment brought in by the phage may be incorporated directly as one of the building blocks. (2) The synapsed segment may also participate in the duplication by attracting replicas of itself, thus

providing a second set of building blocks for the region involved. In the formation of the new chromosome, blocks attracted either by the duplicating chromosome or by the transducing segment may be used, thus incorporating into the new chromosome regions of the chromosome of the donor bacterium by the process suggested by Belling (1933) to explain the mechanism responsible for crossing over.

The results of our experiments with linked genes favor the second possibility. In these experiments we used recipient bacteria of the genetic constitutions *tryA-8 cysB-12* and *cysB-12 tryD-10*, and phage raised on *cysB-14* bacteria. As already mentioned, the *tryA*, *cysB*, and *tryD* loci are linked, and the position of *cysB* is between the two *try* loci. Moreover, *cysB-12* and *cysB-14* are allelic, but recombinations between them do occur. Now, in transduction experiments between *tryA-8 cysB-12* bacteria and *cysB-14* phage a class of bacteria not having any of these three genes (a wild-type class) has been obtained; and this could happen on the basis of assumption (1) only if *cysB-14* were not located between *tryA-8* and *cysB-12*, but to the right of *cysB-12*, since only in that case would a single transducing segment covering *tryA-8* and *cysB-12* not carry *cysB-14*. If this were true, *cysB-14* would be located between *cysB-12* and *tryD-10*, and transduction of *cysB-12 tryD-10* bacteria by *cysB-14* phage would not be expected to produce a wild-type class. The fact that such a class was obtained makes it appear probable that assumption (1) is not valid, and that the mechanism outlined in (2) operates in transduction.

Thus the results of our experiments indicate that the chromosomal segment introduced by phage into a recipient bacterium is small, and that it is incorporated into the bacterial chromosome by a process similar to crossing over.

## SUPPRESSOR MUTATION

Yura has developed a general technique which makes it possible to demonstrate the

occurrence of suppressor mutations in *Salmonella*.

*Genetic heterogeneity of revertants.* When three allelic mutants of *S. typhimurium* requiring adenine plus thiamine for growth (*athA*-1, -2, and -3) were irradiated with ultraviolet (400 ergs per mm<sup>2</sup>) and plated on enriched minimal agar, three types of revertants clearly distinguishable as to colony size were obtained after 6 days' incubation at 37° C (L, large; M, medium; S, small). The allelic mutants all showed this pattern of reversion, and there were no significant differences between them in frequencies of spontaneous

that two independent suppressor mutations at loci *m* and *s* were responsible for the M and S reversion types, respectively, and a back mutation at the *athA* locus for the L type. All the L types obtained by transduction were phenotypically similar to UV-induced L type or wild type. The M' type was different from the M type in that it formed smooth colonies.

When UV-irradiated S-type cells were plated on enriched minimal agar, large and medium colonies were obtained. Since the large colonies were again similar to wild-type colonies, and the medium colonies were smooth like the M' type obtained

TABLE 6

RESULTS OF TRANSDUCTION AMONG THE REVERTANTS, SHOWING PHENOTYPES (COLONY TYPES ON ENRICHED MINIMAL AGAR) AND POSTULATED GENOTYPES

| STRAIN TREATED<br>(GENOTYPE AND<br>PHENOTYPE)                 | TRANSDUCING PHAGE FROM   |      |   |      |   |      |
|---|--|------|---|------|---|------|
|   | Wild and L<br><i>athA</i> <sup>+</sup> <i>m</i> <sup>+</sup> <i>s</i> <sup>+</sup> |      | M<br><i>athA</i> <i>m</i> <i>s</i> <sup>+</sup> |      | S<br><i>athA</i> <i>m</i> <sup>+</sup> <i>s</i> |      |
|   | Gen  | Phen | Gen   | Phen | Gen   | Phen |
| <i>athA</i> <i>m</i> <sup>+</sup> <i>s</i> <sup>+</sup> ..... | <i>athA</i> <sup>+</sup> <i>m</i> <sup>+</sup> <i>s</i> <sup>+</sup>               | L    | <i>athA</i> <i>m</i> <i>s</i> <sup>+</sup>      | M    | <i>athA</i> <i>m</i> <sup>+</sup> <i>s</i>      | S    |
| <i>athA</i> <i>m</i> <sup>+</sup> <i>s</i> , S .....          | <i>athA</i> <sup>+</sup> <i>m</i> <sup>+</sup> <i>s</i>                            | L    | <i>athA</i> <i>m</i> <i>s</i>                   | M'   |   |      |
| <i>athA</i> <i>m</i> <i>s</i> <sup>+</sup> , M .....          | <i>athA</i> <sup>+</sup> <i>m</i> <i>s</i> <sup>+</sup>                            | L    |   |      |   |      |
| <i>athA</i> <i>m</i> <i>s</i> , M' .....                      | <i>athA</i> <sup>+</sup> <i>m</i> <i>s</i>   | L    |   |      |   |      |

or UV-induced revertants of each of the three types. The L type was not detectably different from the wild type, but the M and S types differed from wild type in their growth rates in minimal medium. The L and S types remained stable through several transfers in broth, but the M type, which formed rough colonies, was unstable and mutated back to the original mutant form. The genetic differences among these revertants were confirmed by transduction experiments, which showed that the transducing phage from each type of cells changed the original mutants to the type corresponding to the source of the phage (table 6, first line).

*Transduction and mutation among the revertants.* Table 6 summarizes the results of transduction among the revertants, postulating the genotypes on the assumption

by transduction, it seemed that transduction and UV-induced mutation brought about the same genetic changes in S-type cells. Furthermore, the large-colony type (*athA*<sup>+</sup> *m* *s*) was obtained by UV-induced mutation from the M' type (*athA* *m* *s*).

*Demonstration of the suppressors.* Demonstration of the suppressors *m* and *s* was carried out by means of transduction experiments, using as donors the L type obtained by mutation from S type (*athA*<sup>+</sup> *m*<sup>+</sup> *s*), the L type derived from M' type (*athA*<sup>+</sup> *m* *s*), and the M' type (*athA* *m* *s*), and as recipients *athA*-2 and S type (table 7). The results are consistent with the assumption that the M- and S-type reversions were due to independent suppressor mutations rather than to mutations of the kind that led to the different allelic states at the originally mutating *athA* locus.



The action of the suppressors *m* and *s* could not be detected in the nonallelic mutants tested, and seemed to be specific to the allelic mutants.

*The generality of the phenomenon.* Similar heterogeneity of UV-induced revertants was observed in 5 other strains, making a total of 8 among the 25 purine-requiring mutants tested. Further studies with revertants derived from one of these strains (*adD-11*) have confirmed the validity of the techniques described above and demonstrated that a suppressor muta-

tion, 5 streptomycin-dependent (*sd-4*) strains were used, 4 of which have an additional requirement for an amino acid. In the course of this work the finding, reported by Demerec, that the effect of a mutagen on genes is specific has been repeatedly confirmed.

*Influence of genetic background.* Evidence was obtained from experiments with the five *sd-4* strains that genetic background has a profound influence on the frequency of induced reversions. These five strains were all derived from the same

TABLE 7

RESULTS OF TRANSDUCTION TESTS DEMONSTRATING THE SUPPRESSOR-MUTATION HYPOTHESIS IN M- AND S-TYPE REVERSIONS

| STRAIN TREATED<br>(GENOTYPE AND<br>PHENOTYPE)                 | TRANSDUCING PHAGE FROM   |      |  |      |  |      |
|---|--|------|--|------|--|------|
|   | L  |      | L  |      | M'   |      |
|   | <i>athA</i> <sup>+</sup> <i>m</i> <sup>+</sup> <i>s</i>              |      | <i>athA</i> <sup>+</sup> <i>m</i> <i>s</i>                           |      | <i>athA</i> <i>m</i> <i>s</i>              |      |
|   | Gen  | Phen | Gen  | Phen | Gen  | Phen |
| <i>athA</i> <i>m</i> <sup>+</sup> <i>s</i> <sup>+</sup> ..... | <i>athA</i> <sup>+</sup> <i>m</i> <sup>+</sup> <i>s</i> <sup>+</sup> | L    | <i>athA</i> <sup>+</sup> <i>m</i> <sup>+</sup> <i>s</i> <sup>+</sup> | L    | <i>athA</i> <i>m</i> <i>s</i> <sup>+</sup> | *    |
|   | <i>athA</i> <i>m</i> <sup>+</sup> <i>s</i>                           | S    | <i>athA</i> <i>m</i> <i>s</i> <sup>+</sup>                           | *    | <i>athA</i> <i>m</i> <sup>+</sup> <i>s</i> | S    |
|   |  |      | <i>athA</i> <i>m</i> <sup>+</sup> <i>s</i>                           | S    |  |      |
| <i>athA</i> <i>m</i> <sup>+</sup> <i>s</i> .....              | <i>athA</i> <sup>+</sup> <i>m</i> <sup>+</sup> <i>s</i>              | L    | <i>athA</i> <sup>+</sup> <i>m</i> <sup>+</sup> <i>s</i>              | L    | <i>athA</i> <i>m</i> <i>s</i>              | M'   |
|   |  |      | <i>athA</i> <i>m</i> <i>s</i>  | M'   |  |      |

\* M-type colonies, which were to be expected from these combinations, could not be detected.

tion is the mechanism of reversion in the case of the small-colony type.

#### REACTION OF GENES TO CERTAIN MUTAGENS

Previous studies by Demerec (Year Book No. 51, 1951-1952, pp. 194-196) compared the mutagenic action of manganous chloride, ultraviolet radiation, and  $\beta$ -propiolactone. To extend the findings reported last year, Glover has investigated the mutagenic action of ultraviolet radiation, X-rays, nitrogen mustard, diepoxybutane, and triethyleneimino triazine on *Escherichia coli*. The strains used were selected from among the 35 amino acid-deficient strains in this laboratory and included deficiencies for ten different amino acids. Three of these strains were derived from a B strain of *E. coli*; the others were derived from a B/r (radiation-resistant) strain. In addi-

Sd-4 stock. The additional amino acid requirement in four of them was ultraviolet-induced. Frequency of induced reversions from streptomycin dependence to nondependence was studied in all five strains, and the results are shown in table 8. It appears probable that the gene *me-1* in the strain Sd-4-49 enhances the frequency of reversions from streptomycin dependence, whereas the genes *cys-1*, *cys-2*, and *try-3* in strains Sd-4-77, Sd-4-73, and Sd-4-55, respectively, decrease the mutability of the same *sd-4* gene, in that order.

Another example of the influence of genetic background on frequency of induced reversions is provided by strains D-84 (*ar-3*), M-3 (*ar-3 leu-3*), and M-4 (*ar-3 try-2*). Strains M-3 and M-4 were obtained from D-84 by additional single-step mutations. It was found that the genes

*leu-3* and *try-2* in strains M-3 and M-4 enhance the mutability of the gene *ar-3* common to all three strains. Further evidence in support of the hypothesis that the genetic background influences the frequency of induced reversions was obtained

(Year Book No. 52, pp. 210-211) it was reported that among 35 nutritional deficiencies investigated, 5 were found to be mutagen stable; that is, it was impossible to increase the frequency of reversions above the spontaneous level by treatment

TABLE 8

INFLUENCE OF GENETIC BACKGROUND ON NUMBER OF REVERSIONS INDUCED AT THE *sd-4* LOCUS IN *ESCHERICHIA COLI*

(UV, ultraviolet radiation, 600 ergs per mm<sup>2</sup>; X-rays, 30,000 roentgens; NM, nitrogen mustard, 0.005 per cent; D, diepoxybutane, 0.02 M; T, triazine, 0.001 per cent)

| GENOTYPE                | STRAIN  | INDUCED REVERSIONS PER 10 <sup>8</sup> SURVIVORS |        |       |       |       |
|-------------------------|---------|--|--------|-------|-------|-------|
|                         |         | UV   | X-rays | NM    | D     | T     |
| <i>sd-4 mc-1</i> .....  | Sd-4-49 | 58,000   | 4,380  | 4,160 | 1,175 | 7,320 |
| <i>sd-4</i> .....       | Sd-4    | 27,100   | 4,250  | 3,990 | 910   | 5,500 |
| <i>sd-4 cys-1</i> ..... | Sd-4-77 | 16,900   | 2,970  | 1,640 | 443   | 4,370 |
| <i>sd-4 cys-2</i> ..... | Sd-4-73 | 5,560  | 1,445  | 880   | 249   | 2,890 |
| <i>sd-4 try-3</i> ..... | Sd-4-55 | 2,340  | 260    | 456   | 77    | 2,250 |

TABLE 9

SPECIFIC MUTAGEN STABILITY AND THE INFLUENCE OF GENETIC BACKGROUND ON MUTAGEN STABILITY

(UV, ultraviolet radiation, 600 ergs per mm<sup>2</sup>, B strains 200 ergs per mm<sup>2</sup>; X-rays, 30,000 roentgens, B strains 20,000 roentgens; NM, nitrogen mustard, 0.005 per cent; D, diepoxybutane, 0.02 M, B strains 0.01 M; T, triazine, 0.001 per cent, B strains 0.0005 per cent)

| GENOTYPE                                | STRAIN | GENE TESTED  | SPONTANEOUS FREQUENCY (PER 10 <sup>6</sup> ) | INDUCED REVERSIONS PER 10 <sup>8</sup> SURVIVORS |        |     |        |     |
|---|--------|--------------|--|--|--------|-----|--------|-----|
|   |        |              |  | UV   | X-rays | NM  | D      | T   |
| <i>ar-1 try-5</i> } .....               | WP-12  | <i>ar-1</i>  | { 0.22                                       | 510  | 39     | 1.2 | 81     | 22  |
| <i>ar-1 try-5</i> <sup>+</sup> }        |        |              | { 0.18                                       | 718  | 0      | 0   | 230    | 11  |
| <i>hi-5 leu-2</i> } .....               | M-1    | <i>leu-2</i> | { 0.28                                       | 1,200  | 12     | 5   | 14     | 0   |
| <i>hi-5</i> <sup>+</sup> <i>leu-2</i> } |        |              | { 0.21                                       | 1,040  | 11     | 8   | 2      | 0   |
| <i>ar-3 try-2</i> } .....               | M-4*   | <i>try-2</i> | { 0.21                                       | 10,700   | 1,160  | 25  | 4,200  | 0   |
| <i>ar-3</i> <sup>+</sup> <i>try-2</i> } |        |              | { 0.16                                       | 249  | 55     | 0   | 0      | 0   |
| <i>ar-3 leu-3</i> } .....               | M-3*   | <i>ar-3</i>  | { 0.94                                       | 2,900  | 220    | 7   | 39,000 | 121 |
| <i>ar-3 leu-3</i> <sup>+</sup> }        |        |              | { 1.42                                       | 1,550  | 110    | 16  | 9,640  | 746 |

\* B strains.

from the experiments on specific mutagen stability described in the next section. The combined evidence supports the concept that mutagens do not act directly on genes, but exert their effects indirectly, probably through changes brought about in the cell metabolism, which in turn affect the genome.

*Specific mutagen stability.* Last year

with MnCl<sub>2</sub>, UV radiation, β-propiolactone, X-rays, or a number of other mutagens available in the laboratory. The other mutagens included in the present survey were also used, but no induced reversions were detected. Certain genes, however, have been found to be specifically stable with respect to certain mutagens. Table 9 illustrates the finding that the two genes



*leu-2* in M-1 and *try-2* in M-4 have specific stability for triazine. The data in table 9 further show that in certain genetic backgrounds a gene that is usually mutagen labile may become mutagen stable. In strain WP-12 the genetic change from *try-5* to *try-5*<sup>+</sup> renders the gene *ar-1* stable for X-rays and nitrogen mustard and decreases its mutability after treatment with triazine. The genetic change from *ar-3* to *ar-3*<sup>+</sup> in strain M-4 decreases the effect of all the mutagens on the mutability of *try-2* and renders it stable with respect to nitrogen mustard and diepoxybutane. The genetic changes *hi-5* to *hi-5*<sup>+</sup> and *leu-3* to *leu-3*<sup>+</sup> in strains M-1 and M-3, respectively, also influence the induced-reversion frequencies of the remaining deficiencies in these strains. It may be reasoned that genetic changes of the type *ar-3* to *ar-3*<sup>+</sup> are not the only changes which have occurred in the tested strains, and that some secondary change—for example, the action of a suppressor—is responsible for the observed specific mutagen stability.

To test this hypothesis, six similar lines each requiring a single amino acid were isolated by spontaneous mutation from the original double mutant. The procedure was repeated for all the strains in which specific mutagen stability under the influence of genetic background had been established. The method will be described by using as an example strain M-4, which has the genetic constitution *ar-3 try-2*. The gene *try-2* in this strain yields 25 and 4200 induced reversions per 10<sup>8</sup> survivors in response to treatment with nitrogen mustard and diepoxybutane, respectively. When, however, we obtained as a result of spontaneous mutation a line (M-4-1) having the genotype *ar-3*<sup>+</sup> *try-2*, it was found that neither nitrogen mustard nor diepoxybutane treatment would increase the mutability of *try-2* above its spontaneous rate. In five other M-4 lines (M-4-2 to M-4-6), obtained in the same way as M-4-1, *try-2* showed the same behavior. Thus it seems probable that in strain M-4 the genetic change from *ar-3* to *ar-3*<sup>+</sup> renders the gene

*try-2* stable with respect to nitrogen mustard and diepoxybutane, since it is unlikely that subsidiary changes leading to the same result could occur simultaneously with a mutation of *ar-3* in every one of the six cases studied. Derivatives of strains WP-12 (*ar-1 try-5*<sup>+</sup>) and M-1 (*hi-5*<sup>+</sup> *leu-2*) were similarly tested, and the results support this hypothesis.

*Spontaneous mutability.* The spontaneous-reversion frequencies for these genes were calculated, and the influence of genetic background on the spontaneous frequencies studied. It will be noted from the data in table 9 that the genetic background has an effect on spontaneous frequency, but that this effect bears no relation, either in degree or in direction, to the effect that genetic background may have on induced-reversion frequency. Similarly, the gross number of mutants per 10<sup>8</sup> surviving cells induced by any mutagen is not correlated with the spontaneous-reversion frequency.

*Comparison of the mutagens.* The mutagens used in this survey were compared among themselves and with those employed by Demerec (Year Book No. 51, 1951-1952, pp. 194-196). To compensate for gross differences in mutagenic potency, comparison was made on the basis of percentage figures. These figures expressed for each gene the number of reversions induced by each mutagen as a percentage of the total effect this mutagen had on all the genes taken together. Mutagens were compared to see whether any correlations in the patterns of induced reversions could be established. Certain pairs of mutagens—for example, MnCl<sub>2</sub> and β-propiolactone, diepoxybutane and triazine, UV radiation and X-rays—showed good correlation, whereas others—MnCl<sub>2</sub> and diepoxybutane, UV and nitrogen mustard—showed poor correlation. Each gene was examined to see whether it mutated with equal facility after treatment with each of the seven mutagens. No gene behaved in this way, but a number of genes responded to approximately the same degree to six of the

seven mutagens, the exception being  $\text{MnCl}_2$ .

#### MODIFICATION OF EFFECTS OF ULTRAVIOLET LIGHT

It is known that environmental factors can alter the biological effects of ultraviolet light, even when applied some time after irradiation. In explanation it has been postulated that ultraviolet light causes an instability in the cell which is probably a result of the initiation of short-lived reversible reactions. Changing the environment influences these reactions, either enhancing or lessening the effect of the irradiation. Kelner has demonstrated that exposure of cells to white light after ultraviolet irradiation partly reverses the killing effect and reduces the mutagenic effect of the latter. Since most of the work on this phenomenon, called photoreactivation, has dealt with its relation to survival, it was believed that a further study should be made of its bearing on mutation, with the hope of learning more about the mechanism of mutagenesis. In this study Fitzgerald investigated the effects of ultraviolet irradiation, photoreactivation, and extension of the period of incubation in the dark before plating, on a number of genes in *Escherichia coli* suitable for the purpose.

A mutant strain of *E. coli*, B/r/Sd-4-55S, which carries genes for tryptophane deficiency (*try-3*) and streptomycin dependence (*sd-4*), was used in most of the work.

The relation between ultraviolet dose and extent of photoreactivation produced by a constant exposure to white light was the subject of preliminary experiments. After a low dose of UV, the white-light treatment caused little reversal of killing, but a significant reduction in number of mutants. With higher doses of UV, the same amount of white light produced an increase in survival, the proportion rising markedly with UV dose. The proportion of reduction of mutants increased less markedly with UV dose.

In other experiments, cells treated with UV in phosphate buffer were kept for various lengths of time in the dark at 37° C and then exposed to intense white light and plated on suitable media for determining numbers of survivors and mutants. In comparison with control platings of UV-irradiated cells kept in the dark, the photoreactivated samples showed more survivors and fewer mutants. The efficiency of photoreactivation decreased as the time interval between UV irradiation and initiation of white-light treatment increased. When the interval was about 6 hours the values for survival were the same for the photoreactivated and dark-control samples, that is, no more photoreactivation occurred after this interval. An interval of 7½ hours resulted in the same mutant values for the photoreactivated and the control samples.

In the controls kept in the dark after UV irradiation, an interesting phenomenon was observed, namely, a decrease in survivors and an increase in the relative number of mutants (i.e., number per 10<sup>8</sup> survivors), which became more marked the longer the cells were kept before plating. When plating was delayed for periods up to 2 hours, the absolute number of mutants increased; after longer periods the number dropped, reflecting the decrease in survival. The relative number of mutants, however, continued to increase and the number of survivors to decrease when plating was delayed for periods up to 8 hours and more. Since both genes of the strain behaved in a similar manner, only *try-3* was studied in most experiments, for technical reasons. This phenomenon of a drop in survival and an increase in mutants resulting from postirradiation delay in plating has been called, for convenience, the "dark effect."

Both temperature and UV dose influenced the magnitude of the dark effect. When the bacteria were kept at 15° or 20° C during the period between irradiation and plating, the effect was not observed, the numbers of survivors and mutants remaining constant for as long as



24 hours. With incubation temperatures of 25°, 30°, or 37° C, the effect was present, increasing with temperature. Preliminary storage of irradiated cells at 5° for 6 hours, before 37° incubation, did not alter the dark effect. After doses of ultraviolet below about 600 ergs, little or no dark effect was noticed. Above 600 ergs the dark effect increased with dose.

Cells washed after irradiation and resuspended in fresh buffer showed an increase in the dark effect as compared with unwashed cells, eliminating the possibility that the effect was caused by an action of ultraviolet on the medium. The effect of various carbon compounds, such as citrate, acetate, lactose, and galactose, was studied, with variable, inconclusive results, except that in galactose-adapted cells the number of mutants produced by UV was more than double that found in unadapted cells, and dropped more rapidly when galactose was added after irradiation than in a buffer control.

Strains other than Sd-4-55S were investigated for the dark effect. M-3, a mutant of strain B which has requirements for leucine (*leu-3*) and arginine (*arg-3*), did not exhibit the drop in survival and increase in mutants characteristic of the dark effect, but rather a two- to threefold increase in survival and decrease in relative number of mutants after a few hours' incubation in the dark. The UV doses that could be used were below the threshold dose for production of the dark effect in strain Sd-4-55S, which is radiation resistant. Two mutant strains of B/r, M-1 and WP-12, each with a double amino acid requirement (*hi-5 leu-2* and *arg-1 try-5*, respectively), did not seem to show the dark effect. Another streptomycin-dependent strain (Sd-4-73), which also has a cystine requirement (*cys-2*), showed the dark effect with respect to survival and reversions to *sd-4*<sup>+</sup>. The reversions to *cys-2*<sup>+</sup> were too few to measure accurately. The absolute number of *sd-4*<sup>+</sup> mutants as well as the number per 10<sup>8</sup> survivors increased with delays in plating up to 4 hours, but there was not much drop in survival until

after 4 to 5 hours. Among the strains studied so far, only streptomycin-dependent strains show the dark effect.

One must consider the possibility that the dark effect may be a result of selective division of mutants or killing of nonmutants. The fact that the effect occurs in buffer and in starved cells makes it unlikely that division is responsible. Furthermore, a reconstruction experiment, in which an artificial mixture of Sd-4-55S and its revertants was irradiated and incubated in the dark, resulted in a constant ratio of revertants to original cells over a period of 9 hours, which is good evidence that selection is not responsible for the dark effect. In another experiment carried out to confirm this, irradiated cells were plated on agar without nutrient for the dark-incubation period, instead of being kept in buffer, and then agar suitable for mutant or survivor assays was layered on. Although fewer cells survived under these conditions, the dark effect was still observed. Presumably, if the dark effect were due to postirradiation division, it would be eliminated by this technique, since each original cell would produce only one colony whether or not it had divided in the interval between irradiation and addition of the nutrient.

Preliminary experiments indicate that peroxides are involved in the postirradiation chain of events responsible for the continuing action of UV. Diethyldithiocarbamate, which inhibits peroxide action, eliminated the dark effect when added after irradiation. Survival was only slightly lower after incubation with this compound in the dark than in an untreated irradiated control which was plated immediately. No postirradiation increase in mutants occurred, and the immediate mutagenic action of the radiation was lessened, since the number of mutants in the treated sample was less than in the untreated irradiated control. In an initial experiment, pyruvate, which decomposes peroxides, partly eliminated the dark effect.

These findings are in agreement with

the following hypotheses: that ultraviolet irradiation induces in cells certain changes that affect their metabolism (primary effect), which in turn influences their genome (mutability) and vital functions (survival); that for some time after irradiation the cell is in an unstable state, during which time the environment can influence the course of events leading to the final state; and that peroxides are involved in one of the UV-induced reactions leading to the continuing aftereffects observed in this work.

#### MUTABILITY IN GROWING BACTERIA

In chemostat experiments carried out by Novick and Szilard with a fast-growing, tryptophane-deficient B strain of *E. coli* resistant to phage T<sub>1</sub>, the frequency of T<sub>5</sub>-resistant mutants increased linearly with time in steady-state populations, indicating the absence of selection. Furthermore, when tryptophane was chosen as the limiting growth factor, the spontaneous-mutation rate per unit time was independent of growth rate up to a "generation time" of 12 hours.

To determine whether these findings exemplify a general rule or represent a rather special case, Moser undertook a study with the chemostat to measure spontaneous-mutation rates both for reversion from nutritional deficiency to nondeficiency and for mutation to phage resistance in different B strains of *E. coli*. Two strains with high reversion frequencies for a biochemical requirement were successfully tested in the chemostat. Strain B/1, tr/f/X, derived from Novick and Szilard's strain B/1t/f by ultraviolet treatment, is deficient for a number of amino acids (X) in addition to tryptophane and shows a high rate of reversion from X when plated on tryptophane-supplemented A medium. This strain grew well in the chemostat under tryptophane control when the lactate medium (F) was supplemented with vitamin-free casein hydrolyzate. The second strain

successfully investigated, W-74, which carries the histidine-deficiency gene *hi-5*, grew well in histidine-supplemented F medium or tris hydroxymethylamino methane, with either nitrogen or phosphorus as the limiting growth factor. The usual plating techniques were applied to determine the frequency of nondeficient revertants and of T<sub>5</sub>-resistant mutants. In the case of reversion from histidine deficiency, the cells were washed and concentrated before plating, and in calculating the frequency of revertants a correction was made for mutants originating on the plates instead of in the growth tube of the chemostat.

The experiments carried out with strains B/1, tr/f/X and W-74 showed clearly that both T<sub>5</sub>-resistant mutants and revertants from nutritional deficiency are exposed to a considerable degree of selection when grown at a reduced rate with either tryptophane, nitrogen, or phosphorus control. The mutation frequency does not increase with the first power of time. Under the conditions of these experiments any curvilinear accumulation of mutants is due solely to mutation and selection, selection being caused by differential growth of mutant and nonmutant.

The exploitation of these curves required a mathematical analysis of the mutation-selection process in steady-state bacterial populations. The particular conditions that prevail in the growth tube of the chemostat allowed a simple approach.

If we define  $k$  as the mean growth rate of the parent cells,  $k_m$  as the mean growth rate of the mutant cells,  $\lambda$  as the forward mutation rate per unit time,  $\lambda'$  as the rate of true back mutations per unit time,  $n$  as the density of the nonmutant population,  $m$  as the density of the mutant population,  $V$  as the total population size in the growth tube of the chemostat,  $w$  as the "wash out" or flow rate, and  $t$  as time, the changes in population density of mutant and nonmutant populations in the growth



tube can be described by the following equations (1, 2, 3):

$$\frac{dn}{dt} = (k - \frac{w}{V} - \lambda)n + \lambda'm \quad (1)$$

$$\frac{dm}{dt} = (k_m - \frac{w}{V} - \lambda')m + \lambda n \quad (2)$$

$$\lambda, \lambda' > 0. \quad (3)$$

If we assume, first, that the density of the mutant population remains a small fraction of the total population density  $T$  (thus,  $m \ll T$ , or  $m \ll n$ ), the number of true back mutations per unit time ( $\lambda'm$ ) is a very small fraction of the number of forward mutations per unit time ( $\lambda n$ ), and

$$\lambda'm = 0. \quad (4)$$

If we assume, further, that actual population "change-overs" do not take place between times  $t=0$  and  $t$  (early stages of chemostat experiments), and that a stationary state is maintained in the growth tube from time  $t=0$  to time  $t$  ( $T \approx \text{const}$ ),

$$w, V, \lambda, \lambda' = \text{const}. \quad (5)$$

Furthermore, under such conditions the concentration ( $c$ ) of the controlling growth factor in the growth tube will not change appreciably with increasing mutation frequency during the course of the experiment, so that the growth rates  $k = f_1(c)$  and  $k_m = f_2(c)$  are maintained at approximately the same values and we can introduce a constant selection coefficient,

$$s = \frac{k - k_m}{k} = \text{const}. \quad (6)$$

On integration, in the particular case in which the mutant is not exposed to positive or negative selection ( $s=0$ ), we obtain as a first approximation for mutation frequency:

$$f(t) = f_0 + \lambda t \quad (7)$$

( $f_0$  = initial mutation frequency). This approximation is identical with the formula given by Novick and Szilard.

In the more general case in which selection operates either for ( $s < 0$ ) or against ( $s > 0$ ) the mutant, we obtain, on integra-

tion with  $m \ll n$ , the accurate expression for mutation frequency,

$$f(t) = f_0 e^{-(sk-\lambda)t} + \frac{\lambda}{sk-\lambda} \left[ 1 - e^{-(sk-\lambda)t} \right], \quad (8)$$

in which  $k \approx w/V$ . In the special case of positive selection (mutant grows slower than nonmutant), a mutation-selection equilibrium will be established when the density of the mutant population reaches a level of

$$m_e = n \frac{\lambda}{sk-\lambda} \quad (9)$$

mutant cells per ml.

It may be of interest to note that the same equations (7, 8) are obtained if we assume a delay in the appearance of spontaneously occurring mutants.

Of the theoretically possible curves derived from equation (8), all types actually were detected in experiments with both the tested B strains. Furthermore, there was precise agreement between the theoretical curves and the empirical data. This made it possible to determine rates of mutation both to T5 resistance and to nutritional nondependence in the more common case in which the bacterial mutant is exposed to selection, thus extending the range of application of the chemostat to the study of bacterial mutability.

Some data obtained in successful chemostat experiments with strain W-74 are summarized in table 10. It appears that under certain conditions an effect of growth rate on mutation rate per unit time can become manifest. Under conditions of nitrogen starvation the rate of spontaneous mutation to T5 resistance in this strain remains constant to a "generation time" of about  $3\frac{1}{2}$  hours. With shorter "generation times," however, the spontaneous-mutation rate per unit time increases in a significant way with increasing growth rate. The pertinence of this finding for our understanding of the structure and dynamics of the genetical material in bacteria is clear; but more detailed evidence will be necessary for a discussion of its theoretical implications.

TABLE 10

CHEMOSTAT EXPERIMENTS WITH STRAIN W-74, MEASURING RATES OF MUTATION FROM SENSITIVITY TO RESISTANCE TO PHAGE T5 AND RATES OF REVERSION FROM HISTIDINE DEFICIENCY TO NONDEFICIENCY (*hi-5*→*hi*<sup>+</sup>)

(F medium; 37° C; controlling growth factor, nitrogen [NH<sub>4</sub>Cl, 30 mg/l]; supplement, D,L-histidine HCl [20 mg/l])

| Mutation                                   | Growth rate<br><i>k</i><br>(per hr) | Total population density<br><i>T</i><br>(× 10 <sup>8</sup> /ml) | Selection coefficient<br><i>s</i> | Mutation rate/unit time/cell<br><i>λ</i><br>(× 10 <sup>-8</sup> /hr) |
|--|-------------------------------------|---|-----------------------------------|--|
| B/+→B/1,5 .....                            | 0.120                               | 3.8   | 1.30 × 10 <sup>-1</sup>           | 1.72   |
|  | 0.284                               | 3.5   | 2.17 × 10 <sup>-2</sup>           | 1.61   |
|  | 0.440                               | 2.2   | 1.35 × 10 <sup>-1</sup>           | 4.9  |
| <i>hi-5</i> → <i>hi</i> <sup>+</sup> ..... | 0.116                               | 3.2   | 8.7 × 10 <sup>-2</sup>            | 2.5  |

#### TESTS OF CHEMICALS FOR MUTAGENICITY

As part of a Chemotherapy Screening Program organized by Dr. Alfred Gellhorn for the American Cancer Society, 27 compounds were tested by Hemmerly for mutagenic effect on *E. coli*. The program was designed to test the biological effects of these compounds, and to attempt to correlate these effects with chemotherapeutic activity in relation to cancer.

Tests of mutagenic effectiveness were based on induction of reversions to non-dependence in two streptomycin-dependent mutant strains, and reversions to prototrophy in several nutritionally deficient strains. All the compounds were tested with the streptomycin-dependent strain Sd-4, and with strain WP-14, which is proline deficient (*pro-1*). In addition, 11 of the compounds were also tested with strain Sd-4-73, which carries a deficiency for either cystine or methionine (*cys-2*) as well as streptomycin dependence (*sd-4*), and with strain WP-2, which has a requirement for tryptophane (*try-6*). In strain Sd-4-73, reversions of both *cys-2* and *sd-4* were scored.

Those compounds that are readily water soluble were dissolved in distilled water. When necessary, the pH was altered or the compound used in the form of a lactate or acetate in order to increase solu-

bility. The compounds that are only slightly soluble in water were shaken mechanically for 30 minutes, after which the supernatant was decanted and used in treatment. Bacterial suspensions were washed in saline, resuspended in appropriate dilutions of the compound to be tested, and incubated at 37° C for 1 or 3 hours. Samples were then assayed to determine the total number of surviving bacteria, and appropriate dilutions were plated in order to find out the number of reversions. The mutagenic effectiveness of a compound was calculated by correcting the number of revertants obtained from treated bacteria for the number obtained in control platings.

The results of this investigation are summarized in table 11. Most of the compounds tested had no mutagenic effect on *sd-4*, but only 6 of the 27 showed no mutagenic activity for *pro-1*. In general, although *sd-4* was much less sensitive than *pro-1*, its reaction appears to be a better indicator of the responses of the other genes.

Slight to fair mutagenic activity was found for the following compounds: amethopterin, urethane, 1,4-dimethanesulfonyloxybutane, and 2,6-diaminopurine. 2,4-Diamino-5-(3',4'-dichlorophenyl)-6-ethyl pyrimidine, deoxypyridoxine, and diamino-



biuret were found to have somewhat greater mutagenic action. Only nitrogen mustard, a standard mutagen, and azaserine, a new antitumor agent, were found to be especially effective. Of these two,

strain Sd-4. Concentrations as low as  $3.1 \times 10^{-5}$  M gave large numbers of induced reversions, but maximal effects were obtained with concentrations above  $6.2 \times 10^{-4}$  M (table 12). When, using a concentration

TABLE 11

SUMMARY OF CONCLUSIONS FROM EXPERIMENTS TESTING INDUCTION OF REVERSIONS IN CERTAIN GENES OF *ESCHERICHIA COLI* TREATED WITH CERTAIN CHEMICALS

(+, mutagenic effect; —, no mutagenic effect; ±, slight mutagenic effect)

| COMPOUND   | STRAIN AND GENE     |             |              |                       |                      |
|--|---------------------|-------------|--------------|-----------------------|----------------------|
|  | Sd-4<br><i>sd-4</i> | Sd-4-73     |              | WP-14<br><i>pro-1</i> | WP-2<br><i>try-6</i> |
|  |                     | <i>sd-4</i> | <i>cys-2</i> |                       |                      |
| Methyl bis(β-chloroethyl)amine hydrochloride (nitrogen mustard) .. | +++                 | +++         | +            | ++++                  | ++                   |
| Amethopterin .....   | ±                   | —           | —            | +                     | +                    |
| Hydrocortisone .....   | —                   | ..          | ..           | ±                     | ..                   |
| N-Methyl formamide .....   | —                   | —           | —            | —                     | —                    |
| N-Methyl acetamide .....   | ±                   | ..          | ..           | +                     | ..                   |
| 6-Mercaptopurine .....   | —                   | ..          | ..           | ±                     | ..                   |
| Urethane .....   | +                   | —           | —            | +                     | —                    |
| Methyl carbamate .....   | —                   | ..          | ..           | ±                     | ..                   |
| Chloramphenicol .....  | —                   | —           | —            | —                     | —                    |
| Potassium arsenite .....   | —                   | —           | —            | —                     | —                    |
| 8-Azaguanine .....   | —                   | ..          | ..           | —                     | ..                   |
| 8-Azaxanthine .....  | —                   | ..          | ..           | ±                     | ..                   |
| Benzimidazole .....  | —                   | ..          | ..           | ±                     | ..                   |
| D-Glucosamine .....  | —                   | ..          | ..           | —                     | ..                   |
| 2,6-Diaminopurine .....  | +                   | —           | —            | +                     | ..                   |
| 1,4-Dimethanesulfonyloxybutane ..                                  | +                   | ..          | ..           | ±                     | ..                   |
| Diethylstilbestrol .....   | —                   | ..          | ..           | —                     | ..                   |
| 2,4-Diamino-5-(3',4'-dichlorophenyl)-6-ethyl pyrimidine .....      | +                   | ..          | ..           | +                     | ..                   |
| 2,4-Diamino-5-(4'-chlorophenyl)-6-ethyl pyrimidine .....           | —                   | ..          | ..           | ±                     | ..                   |
| Diaminobiuret .....  | +                   | +           | ++           | +                     | +                    |
| Ethionine .....  | —                   | ..          | ..           | —                     | ..                   |
| β-Thienylalanine .....   | —                   | ..          | ..           | —                     | ..                   |
| Deoxypyridoxine .....  | +                   | ..          | ..           | +                     | ..                   |
| Colchicine .....   | —                   | ..          | ..           | ±                     | ..                   |
| 5-Methoxytoluquinone .....   | —                   | ..          | ..           | —                     | ..                   |
| Netropsin .....  | ±                   | ..          | ..           | +                     | ..                   |
| Azaserine .....  | ++++                | ++++        | ++++         | ++++                  | ++++                 |

azaserine was much more mutagenic than nitrogen mustard for all the loci tested except *pro-2*. With this group of strains, azaserine was found to be as effective as and in some cases more effective than ultraviolet irradiation or treatment with manganous chloride.

More detailed studies of the mutagenic action of azaserine were carried out with

of  $6.2 \times 10^{-4}$  M, the length of time of treatment was varied, an appreciable number of induced revertants was found after as little as 5 minutes' treatment at 37° C; and frequency of mutants increased with longer treatments up to 90 minutes, after which the values leveled off (table 13). By adjusting the concentration and the length of treatment, large numbers of mutants were

TABLE 12

EFFECT OF VARYING THE CONCENTRATION OF AZASERINE ON FREQUENCY OF INDUCED REVERSIONS IN STRAIN Sd-4

(Treated suspensions incubated for 90 minutes at 37° C)

| Concentration (M)            | No. induced reversions per 10 <sup>8</sup> viable bacteria | Per cent survival |
|------------------------------|--|-------------------|
| 3.1 × 10 <sup>-5</sup> ..... | 1680   | 73.3              |
| 6.2 × 10 <sup>-5</sup> ..... | 3950   | 75.8              |
| 3.1 × 10 <sup>-4</sup> ..... | 4350   | 73.3              |
| 6.2 × 10 <sup>-4</sup> ..... | 5680   | 73.3              |
| 1.6 × 10 <sup>-3</sup> ..... | 5970   | 26.1              |
| 3.1 × 10 <sup>-3</sup> ..... | 6300   | 9.8               |

TABLE 13

EFFECT OF LENGTH OF TREATMENT WITH AZASERINE AT 37° C ON FREQUENCY OF REVERSIONS IN STRAIN Sd-4

(Concentration, 6.2 × 10<sup>-4</sup> M)

| Time (min) | No. induced reversions per 10 <sup>8</sup> viable bacteria | Per cent survival |
|------------|--|-------------------|
| 5.....     | 620  | 100               |
| 15.....    | 1,150  | 89.9              |
| 30.....    | 2,760  | 100               |
| 60.....    | 10,900   | 38.4              |
| 90.....    | 11,600   | 22.9              |
| 120.....   | 9,200  | 15.1              |
| 150.....   | 9,900  | 7.3               |
| 180.....   | 10,600   | 3.8               |
| 210.....   | 11,500   | 2.0               |

obtained with relatively little killing, since a large part of the killing action of azaserine does not take place until after the number of induced revertants has begun

to reach its maximal level. Thus, a large increase in the absolute number of mutants can be observed. Such increases are not usually found after treatment with mutagenic agents.

Some experiments were also done to test the effect of varying the temperature at which treatment was carried out. At temperatures below 10° C, relatively few mutations were induced and there was little increase in the number after long

TABLE 14

FREQUENCY OF INDUCED REVERSIONS IN STRAIN Sd-4 WHEN O-DIAZOACETYL-D-SERINE WAS USED INSTEAD OF O-DIAZOACETYL-L-SERINE

(Suspensions incubated for 60 minutes at 37° C)

| Concentration (M)            | No. mutants per 10 <sup>8</sup> viable bacteria | Per cent survival |
|------------------------------|---|-------------------|
| 6.2 × 10 <sup>-5</sup> ..... | 123   | 73.3              |
| 6.2 × 10 <sup>-4</sup> ..... | 556   | 67.8              |
| 3.1 × 10 <sup>-3</sup> ..... | 630   | 43.4              |
| 6.2 × 10 <sup>-3</sup> ..... | 2080  | 17.6              |

periods of treatment. Above 15° C, increasing the temperature also increased the number of mutants, particularly in the first 15 to 30 minutes of treatment. With longer periods of treatment, the differences in number of mutants between samples held at the various temperatures were much less.

All the results reported for azaserine were obtained with O-diazoacetyl-L-serine. A sample of O-diazoacetyl-D-serine, made available by Dr. C. Chester Stock of the Sloan-Kettering Institute for Cancer Research, was also found to be mutagenic, but at a much lower level (table 14).

## BACTERIAL GENETICS—II

EVELYN M. WITKIN AND ANN M. LACY

### KINETICS OF TRANSDUCTION

In October 1953, we began studies on some aspects of the kinetics of transduction in *Salmonella typhimurium*. Described

first by Zinder and Lederberg, transduction is now understood to be a mechanism whereby heritable characteristics of a strain are transferred, usually singly, to a



variable proportion of the members of a genetically different but related strain, through a bacteriophage vector. Except for the currently apparent differences in the nature of the active agent, transduction is sufficiently similar to bacterial type transformation to suggest that the two mechanisms may be basically the same. Together, they constitute an important source of genetic variability, and although it is not yet possible to assess the relative weights of these phenomena, the study of transduction and transformation has taken a place beside that of mutation and sexual recombination in the over-all investigation of bacterial heredity.

Our work this year has centered on the timing of some of the critical events in transduction, in an effort to learn when and how the genetic material carried by bacteriophage from the donor strain is incorporated into the working genotype of the recipient strain. Using three related experimental approaches, we have attempted to determine, first, the number of clones derived from transduced cells appearing as a function of the multiplication of the population infected with transducing phage (delay experiments); second, the relative rates of increase in the size of clones derived from transduced and nontransduced cells in the same population in the early divisions following infection with transducing phage (respreding experiments); and, third, the ultimate genetic composition of clones containing the descendants of transduced cells (replicating experiments).

*Delay experiments.* In these experiments, we used six auxotrophic substrains derived from the wild-type phage-sensitive strain LT2 (obtained through the courtesy of N. Zinder) by the penicillin method. These strains carry requirements for: histidine (*hi-23*), proline (*pro-14*), lysine (*ly-2*), cysteine (*cys-18*), adenine (*ad-5*), and tryptophane (*try-3*). The experiments involved mixing temperate phage grown on the wild-type bacteria with washed suspensions of each auxotroph, allowing

adsorption to take place, and plating the mixtures on minimal or semi-enriched minimal medium in such a way as to obtain within each experiment a controlled range of residual divisions of the plated population. In a given experiment, the range of residual divisions was typically from 0 to 8, and was controlled precisely by varying the inoculum size, or the degree of limiting enrichment of the medium, or both. The extent of residual growth was determined directly by the plate-washing method after 48 hours of incubation. Parallel platings of the bacterial suspensions without phage gave the spontaneous-reversion values, which were in all cases negligible as compared with the number of prototrophs originating through transduction.

We have found that the rate at which clones derived from transduced cells appear, as a function of the multiplication of the infected population, differs for different mutants, as shown in figure 7. In some cases (*hi-23*, *ad-5*), almost as many effective transductions occur on plates showing no residual growth of the infected population as on plates showing one or more divisions. In other cases (*pro-14*, *cys-18*, *ly-2*), there is a gradual increase in the number of effective transductions with increasing multiplication of the inoculum, the number reaching a stable maximum in about 3 to 4 generations. In one case (*try-3*), very few transductions are detected when the infected population undergoes no multiplication, but the maximum yield is obtained when the initial population is permitted to divide once. Our interpretation of these results will be discussed below, after the presentation of other data.

*Respreding experiments.* Using Newcombe's respreding technique, we have determined for three strains (*hi-23*, *try-3*, *ly-2*) the clonal growth rates of transduced and nontransduced cells in the early divisions following infection with transducing phage. Infected populations were plated on minimal medium sufficiently enriched with nutrient broth to allow the

maximum residual growth required for full expression of transductions. The growth rate of the population as a whole was determined by plate-washing at intervals from 0 to 6 hours. The growth rate of clones derived from transduced cells was determined by resspreading the surface of several plates at the same time intervals to break up microcolonies and redistribute the individual cells as new centers of colony growth. High multiplicities of infection were used, so as to minimize the amount of lysis occurring after infection.

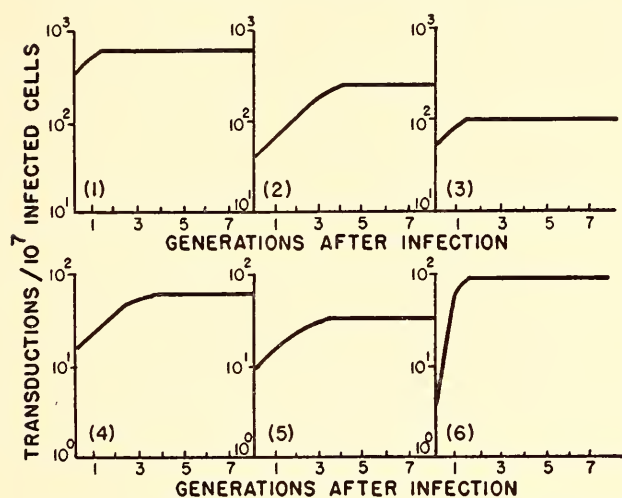


FIG. 7. Delayed appearance of prototrophs arising by transduction. (Multiplicity of infection, ca. 5.) (1) Histidineless (*hi-23*), (2) cysteineless (*cys-18*), (3) adenineless (*ad-5*), (4) prolineless (*pro-14*), (5) lysineless (*ly-2*), (6) tryptophaneless (*try-3*).

It was found that, in all three strains, clones derived from nontransduced cells initiate multiplication earlier than clones descended from transduced prototrophic cells, and this earlier onset of multiplication is reflected in a persistent lead of 1 to 2 generations, since the growth rate of transduced and nontransduced cells is the same once multiplication begins. The fact that clones derived from transduced cells are smaller than clones derived from unmodified cells during logarithmic growth can be accounted for by either of two possible explanations: (1) transduced cells have a longer lag phase than nontransduced cells, and therefore start to multiply later; or (2) cells giving rise to clones modified

by transduction begin to multiply at the same time as other cells, but the incorporation of the new genetic material is delayed, and occurs in only a fraction of the daughter cells, ultimately yielding a genetically mixed clone. Our third experimental approach was undertaken in an effort to distinguish between these two possibilities.

**Replica-plating experiments.** In order to determine whether clones in which transduction has occurred are composed purely of prototrophs or are genetically mixed, containing also unaltered cells, it was necessary to isolate such clones without selection, under conditions permitting the survival and maximal multiplication of both components of a possible mixed clone. Transducing phage was mixed with a suspension of the histidineless strain, and a suitable dilution of the mixture was plated on nutrient agar so as to obtain about 500 well separated colonies after overnight incubation. Using the method of Lederberg, Zinder, and Lederberg, sterile velvet pads held taut in a rigid frame were used to replicate the colonial topography of these plates on the surface of minimal medium. After 8 hours of incubation, the replica plates were examined, and any colonies appearing on them served to locate, on the original nutrient agar plates, the colonies presumably containing prototrophs descended from transduced cells. These were carefully picked and analyzed to determine whether they consisted purely of prototrophs, or represented mixtures of prototrophs derived from transduced cells and the auxotrophic parental type. Eleven colonies in which transduction had occurred, and which therefore contained prototrophs, were isolated by this method, and all eleven colonies were found to be mixed clones, containing also the parental auxotroph (*hi-23*). The percentages of prototrophs in the eleven colonies were as follows: 83, 52, 40, 32, 31, 24, 15, 9, 7, 0.01, 0.01. Of course, the proportions of the two components are subject to change during the growth of the colony as a result of



selective factors, and it is therefore not justifiable to use these values as a basis for reconstructing the timing of the genetic fixation of the character changed by transduction. Taken together with the results of the resspreading experiments, these results suggest that in transduction the altered character is genetically fixed in only a fraction of the clone arising from the infected cell, most often at the time of the first or second division.

Our results bear on the mechanism of incorporation of the material borne by the transducing phage into the working genome of the recipient cell. Two general possibilities can be postulated: (1) that the fragment of genetic material from the donor cell is itself physically incorporated into the recipient chromosome, actually replacing its homologue; or (2) that the fragment brought in by the transducing phage is not actually incorporated into the recipient chromosome, but serves as a more or less persistent model for the reduplication of the chromosomal area in question. It is not likely that these hypotheses can easily be resolved, but our results make it possible to eliminate the following particular versions of each of them: (a) that the fragment is physically incorporated into the recipient chromosome before duplication (assuming that the chromosome is in a single-strand condition), since this would lead to pure clones of prototrophs derived from transduced cells; and (b) that the fragment serves continuously as a model for reduplication for more than one or two generations, since this would lead to pure clones or to mixed clones containing a very small fraction of auxotrophic bacteria derived from nontransduced cells.

Underlying our interpretation of these experiments is the assumption that resting cells of *Salmonella* are not typically multinuclear. Segregation of nuclei could account for the mixed clones, without necessarily bearing on the mechanism of incorporation. Similar cultures of *Escherichia coli*, however, are known to be predominantly uninuclear, and the degree of rela-

tionship between the two species makes our assumption reasonable. We intend, however, to test it through cytological examination and the use of a double-screening procedure.

*Dominance in transduced cells.* We are now in a position to assess the significance of the delay experiments described earlier. These experiments differ from the resspreading and replica-plating experiments in that they test the *eventual* appearance or nonappearance of clones derived from transduced cells as a function of the amount of multiplication undergone by the infected population as a whole, and give us no direct information regarding the age or size of such clones as compared with that of clones derived from nontransduced cells. In the case of *hi-23*, the results of the replica-plating and resspreading experiments indicate clearly that the genetic fixation of the changed character typically is delayed one to two divisions after infection, yet the delay experiments show a large fraction of clones derived from transduced cells appearing when the infected population is not allowed to multiply at all. This seeming contradiction can be resolved by introducing the concept of dominance. If we assume that an infected cell, into which the wild-type locus has been carried by the transducing phage, is rendered capable of multiplication on minimal medium even before the locus in question is incorporated, by virtue of its ability to exert dominance under such conditions, we can account for the apparent "zero points" in transduction. In other words, it is not necessary to infer that absence of multiplication of the infected population as a whole is always an indication that cells carrying the wild-type locus brought in by the transducing phage are also unable to multiply. Evidence in support of this has been found by Stocker, Zinder, and Lederberg (1953), and its importance lies not merely in providing an explanation for an apparent contradiction in these experiments. Expression curves, relating the rate of appearance of mutants

to the number of divisions of a large population, have been used widely in the study of induced mutations, and inferences have been made as to the timing of events concerned with the mutation process. In some cases, the expression curves may measure primarily the degree of dominance of the induced mutation.

#### DELAYED APPEARANCE OF INDUCED MUTANTS

Populations of bacteria treated with mutagens must usually be permitted to pass through a variable number of divisions before the full yield of induced mutations can be detected. The basis of the delayed appearance of induced mutants is not yet fully understood. We have conducted a series of experiments in which transduction in *Salmonella* was used as a tool to obtain further information about this phenomenon.

In the study of the kinetics of transduction described in the previous section, it was found that prototrophs arising by transduction are characterized by a delayed appearance formally analogous to that of induced mutants: the population infected with transducing phage must be permitted to multiply one or more times before the full yield of transduced cells can be determined. We have compared the patterns of delayed appearance of prototrophs arising by transduction and of ultraviolet-induced prototrophs derived from the four auxotrophic strains requiring, respectively, proline, lysine, adenine, and tryptophane. For transduction, washed suspensions of bacteria were mixed with phage grown on the wild type; for ultraviolet induction, washed suspensions were irradiated with 300 ergs per mm<sup>2</sup> of ultraviolet light (survival 10 per cent). Delayed appearance of transduced and induced prototrophs was measured by the same method, that of plating on minimal or semi-enriched minimal medium so as to obtain a controlled range of residual divisions within each experiment. In the case of *ad-5*, additional experiments were made in which bacteria irradiated with

300 ergs per mm<sup>2</sup> of ultraviolet light were infected with transducing phage under conditions permitting the detection of prototrophs arising by transduction but not those arising by induction.

The extent of delay was found to be consistently greater in the case of ultraviolet-induced mutation than in that of transduction. Table 15 shows the number of generations required after treatment with transducing phage or ultraviolet light to detect the full complement of prototrophs arising by transduction or induction. The patterns of delay for both transduction and

TABLE 15

DELAYED APPEARANCE OF PROTOTROPHS PRODUCED BY TRANSDUCTION AND ULTRAVIOLET INDUCTION

| Strain              | Transduction delay<br>(generations) | UV-induction delay<br>(generations) |
|---------------------|-------------------------------------|-------------------------------------|
| <i>ad-5</i> .....   | 1-2*                                | 5-6                                 |
| <i>pro-14</i> ..... | 3-4                                 | 5-6                                 |
| <i>try-3</i> .....  | 1                                   | 7-8                                 |
| <i>ly-2</i> .....   | 3-4                                 | 12-13                               |

\* Unchanged when bacteria are irradiated before transduction.

ultraviolet induction seem to be stable features of the individual locus, and are not affected by factors that alter the absolute number of prototrophs obtained, such as multiplicity of infection or dose of ultraviolet.

In interpreting these results, it must be remembered that transduction does not involve mutagenesis, but is rather a process of transfer of genetic material from one strain to another—in short, a kind of recombination. Thus, certain of the possible mechanisms that have been postulated to account for induction delay should operate also in transduction delay, whereas others—those arising from the special properties of the newly induced mutant cell—should not. Both systems should be subject to delay due to nuclear segregation, phenotypic lag, irregularity of onset of division in irradiated populations (if irradiated



cells are used for transduction, as was done with *ad-5*), and systematic errors in the method of detecting prototrophs. These factors cannot account for the difference in magnitude of delay, although one or more of them may be represented in the common residue. Thus, the major factors in the delayed appearance of induced mutants

appear to be those that depend upon the special properties of the newly induced mutant cell: delayed mutation (Witkin, 1951; Newcombe, 1953) and selectively delayed onset of division of newly induced mutant clones (Ryan, 1954). Experiments designed to assess the relative roles of the two final possibilities are in progress.

## ORGANIZATION OF CELLULAR MATERIALS

B. P. KAUFMANN, M. R. McDONALD, M. H. BERNSTEIN, H. GAY, C. GHOSH, G. R. KERSH,  
AND J. R. GHEE

During the past year we have continued our efforts to obtain the information about patterns of organization of cellular materials that is essential to an understanding of the nature and specificity of action of the gene. Attention has been focused on the nucleoprotein complexes of the normal cell and the changes effected therein by experimental procedures. The methods of analysis employed have included enzymatic hydrolysis of fixed cells in tissue sections, and assay of materials extracted from cells or isolated nuclei. It has thus been possible to interpret the morphological changes disclosed by methods of descriptive cytology in terms of alterations in composition of essential structural components.

Within recent years descriptive cytology has moved into a new and hitherto unexplored realm of fine detail, formerly known as the level of submicroscopic morphology. Developments in techniques for electron microscopy have made this advance possible; details of structure that were not discernible with the ordinary light or phase-contrast microscope now appear with brilliant clarity in electron micrographs. The chemical nature and organization of these newly discovered cell structures remain to be determined, and we are now utilizing for this purpose the cytochemical methods which have engaged our attention during recent years. In attempting to keep abreast of developments in this field, we have continued co-operative efforts with Dr. Thomas F. Anderson of the Johnson Foundation of the University

of Pennsylvania. Working in Dr. Anderson's laboratory, Miss Helen Gay has developed a method of obtaining ultrathin serial sections of intact cells and smears that should immeasurably facilitate efforts to construct three-dimensional models showing distribution and interrelations of nuclear and cytoplasmic particles of extremely small dimensions.

Analyses of fixed cells and cellular extracts have afforded important clues to an understanding of the organization of the materials that constitute the living cell. In an effort to obtain such information by more direct methods, we envisioned a few years ago the use of the television microscope for quantitative spectrophotometry of the living cell. Adaptation of the microscope for such studies was worked out with staff members of the Brookhaven National Laboratory. The equipment, formerly on loan from the Radio Corporation of America, has recently been acquired by the Biology Department at Brookhaven. Collaborative efforts in the study of biological problems will continue as heretofore with Dr. Montrose J. Moses.

Throughout the year we have utilized temporary assistance from time to time. Workers who have served on this basis include Lois M. Towers, Sydney Smith, Suzanne Hume, Lucy Pederson-Krag, and Anders J. Kaufmann.

Our research program has again been facilitated by a grant (RG-149) from the National Institutes of Health, U. S. Public Health Service.

## CYTOCHEMICAL STUDIES

*Action of ribonuclease on apurinic acid.*

The validity of conclusions about patterns of organization based on experiments involving enzymatic hydrolysis depends on the specificity of action of the enzymes used. Because of this requirement, considerable effort has been devoted during recent years to the preparation and careful assay of the enzymes used in our experiments. These purified enzymes are highly specific; ribonuclease, for example, was found to hydrolyze ribonucleic acid (RNA) but not deoxyribonucleic acid (DNA), whereas deoxyribonuclease was found to degrade DNA but not RNA.

Despite these results, and the confirmation afforded by work in other laboratories, the question of specificity of enzymatic action, particularly of ribonuclease, still arises from time to time. For example, as noted in Year Books Nos. 50 and 51, treatment of sections of onion root tips with ribonuclease at 60° C and pH 6 decreased their stainability by the Feulgen procedure. The decrease was found to be due to the degradation of DNA by a deoxyribonuclease, present in the onion root-tip sections, which can hydrolyze isolated DNA but cannot hydrolyze intracellular DNA unless the cells have been previously treated with ribonuclease. More recently, Durand and Thomas have shown that after hydrolysis of tissue sections in 1 N hydrochloric acid the nuclei stain red instead of green-blue with Unna's methyl green-pyronin mixture. Exposure of such acid-hydrolyzed sections to ribonuclease rendered them unstainable with pyronin or the leucobasic fuchsin of the Feulgen procedure. Similar results were obtained with spots of isolated DNA which had been dried, fixed in Zenker's or Helly's fluid, and then hydrolyzed with 1 N hydrochloric acid. These investigators showed that this sensitization of DNA to degradation by ribonuclease is due to the liberation of purine bases from DNA by the acid hydrolysis, with resulting formation of apurinic acid. They suggested that

ribonuclease hydrolyzes the esters, not only of D-ribose-b-phosphate and of certain glycosides such as pyrimidine-b-nucleotides, but also of substances like D-2-deoxyribose-3(or 5)-phosphate.

During the past year McDonald and Kaufmann have confirmed Durand and Thomas' findings both chemically and cytochemically, but have found that this degradation of apurinic acid is not a specific property of ribonuclease. Similar results are obtained with many proteins, including lysozyme, cytochrome c, histone,

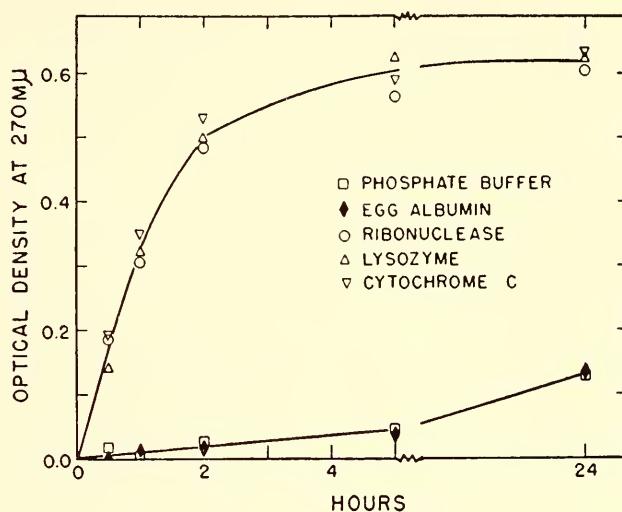


FIG. 8. Degradation of apurinic acid by various proteins. Increase in optical density indicates extent of degradation.

chymotrypsin, chymotrypsinogen, and trypsin. In fact, of the proteins tested thus far, negative results have been obtained only with egg albumin and serum albumin. Several representative examples are shown in plate 1 (A-D) and figure 8. Degradation of apurinic acid by the previously mentioned proteins appears to be a function of the basicity of the proteins, the most basic ones, such as lysozyme, cytochrome c, and ribonuclease, being the most effective. The reaction is a property of the native protein molecule, since denaturation of the protein results in loss of its degradational capacity. The rate of degradation is proportional to the concentration of protein used, whereas the extent of degradation is independent of the protein concentration. The rate of degradation is



temperature dependent. It is a function also of the pH of the reaction medium, being directly proportional to pH in the range from 4.1 to 6.9 for ribonuclease, and from 4.1 to 8.0 for lysozyme. Above these values the rate is independent of pH in the range thus far studied (fig. 9). These limiting pH values appear to be related to the basicity of the protein used; the isoelectric points of ribonuclease and lysozyme, as determined electrophoretically by the moving-boundary method, are 9.5 and 11.0, respectively.

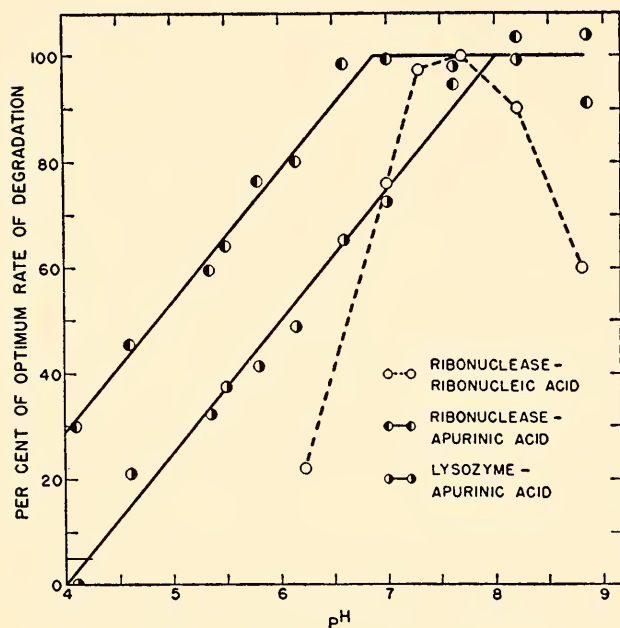


FIG. 9. Effect of pH on rate of degradation of apurinic acid by basic proteins.

Apurinic acid is apparently an unstable molecule. Tam and his co-workers have shown that it is slowly degraded by magnesium ions (0.02–0.2 M). Degradation by basic proteins is a much faster process, the reaction (as measured by the formation of dialyzable products) being complete in 24 hours at 37° C with concentrations of protein of the order of  $2 \times 10^{-4}$  M.

*Action of ribonuclease in producing mitotic disturbances.* Immersion of growing roots of onion and lily in aerated solutions of ribonuclease leads to production of mitotic abnormalities in the meristematic cells (Year Book No. 52). Adhesion of chromosomes, swelling of nuclei and

nucleoli, reversal of mitotic phase, and impairment of mitotic mechanisms—all of which are produced by the action of ribonuclease on the living cell—may be attributed, on the basis of information obtained in our studies, to changes in the gel-like properties of constituent nucleoproteins.

The types of aberration listed above are similar to those that have been designated as “primary effects” when induced by ionizing radiations. We have suggested accordingly that these primary effects can be initiated by changes in RNA. The question then arises whether the chromosome breaks and structural rearrangements that characterize the “secondary effects” of ionizing radiations can also be produced by the action of ribonuclease.

In an effort to answer this question, Kaufmann and Kersh exposed adult males of *Drosophila melanogaster* to an aerosol of ribonuclease, using equipment and methods of treatment that had proved effective in studies of the action of nitrogen mustard (Year Book No. 47, 1947–1948), to determine whether reciprocal translocations between the second and third chromosomes would be produced. The treated males were mated with females carrying the dominant markers Curly, Plum, Hairless, and Stubble. Heterozygous F<sub>1</sub> flies were mated individually with unrelated wild-type flies of the opposite sex, and the F<sub>2</sub> cultures examined for the presence or absence of chromosomal rearrangements. No induced translocations were found among 1478 cultures tested. Chemical assay showed that the aerosol retained at least 85 per cent of the activity of the original solution, and therefore the negative results indicate either that the enzyme failed to reach the spermatozoa, or that it was unable to produce chromosome breaks. The aerosol had no significant toxic effect on the flies, since survival was more than 90 per cent in all treatments. In related tests with *Bacillus megaterium*, Dr. W. Szybalski, of the Biological Laboratory, found that high concentrations of ribonu-

clease (10 mg per ml agar substrate) did not inhibit growth or division. Any conclusions about the nature of "structural" changes drawn from these experiments must be regarded as tentative, but it appears probable that more profound alterations are required to produce structural changes than to produce those of the primary type.

Further evidence of the degradational action of ribonuclease in the living cell is afforded by the release of deoxyribonuclease from growing roots immersed in aqueous solutions of ribonuclease, as was reported preliminarily in Year Book No. 52. No deoxyribonuclease was released into the medium when roots were immersed in control solutions that do not degrade RNA, including inactivated ribonuclease, egg albumin, tap water, sucrose, and colchicine. The presence or absence of deoxyribonuclease in the culture medium was determined by its effect on the stainability of tissue sections by methyl green, orcein, or the Feulgen reagent. Since onion root-tip deoxyribonuclease will not degrade intracellular DNA unless the sections have been hydrolyzed in ribonuclease, it was necessary to pretreat with ribonuclease those tissue sections that were exposed to the action of the control solutions, or to add ribonuclease to the control solutions. When used in combination with ribonuclease in either of these ways, none of the control solutions in which roots had been growing altered the stainability of DNA in tissue sections. The deoxyribonuclease released from the root-tip cells by the action of ribonuclease did not degrade the DNA in those cells, as was determined by cytochemical studies of sections of roots that had been growing in ribonuclease. This inability to degrade intracellular DNA was probably due to the fact that treatments were carried out at room temperature. Previous studies had shown that depolymerization of DNA by onion root-tip deoxyribonuclease is greatest at 60°–70° C (Year Book No. 51).

*Production of mitotic abnormalities by*

*cytochrome c.* Evaluation of the experiments reported in Year Book No. 52, in which growing roots were treated with ribonuclease, indicated that the terminal phases of the reaction, as evidenced by the production of abnormalities, might conceivably be reached through a number of different channels, including degradation of either the nucleic acid or the protein component of nucleoproteins, and functional impairment of enzyme systems associated with RNA-containing structures.

In an attempt to qualify this generalization, experiments were undertaken during the past year by Kaufmann and Ghosh in which solutions of cytochrome c were used in treatment of onion roots. There was some prospect that this enzyme could enter the living cell, since it has a molecular weight of about 13,000 (essentially that of ribonuclease), and is soluble in water. Cytochrome c is a heme protein which functions in the living cell as an intermediate carrier between molecular oxygen and systems such as cytochrome-c reductase. Because of its importance in cellular respiration, it seemed probable that an excess of cytochrome c in the living cell might interfere with normal metabolic processes.

Growing roots of onion were immersed in both aerated and nonaerated solutions of this enzyme. (The equipment used for aeration is illustrated in the *Pfizer Spectrum* for February 20, 1954, as part of a pictorial survey of the work of this Department.) Nonaerated solutions (0.5 mg of cytochrome c per ml of distilled water at pH 6) caused excessive contraction and stickiness of chromosomes, and some *c*-mitoses and polyploid cells. The effects of aerated solutions were less severe. As with ribonuclease, the over-all effects and the potentialities for recovery were dependent on the concentration of the enzyme and the time of treatment. Another parallelism with the action of ribonuclease was the ability of cytochrome c to reduce pyronin stainability and increase fast-green stainability.



bility in tissue sections. Boiled cytochrome c did not have this ability. Chemical assay showed that the sample of enzyme used did not degrade isolated RNA. Preliminary experiments indicate that the reduction in pyronin stainability and the increase in fast-green stainability effected by cytochrome c is probably due to combination of this basic protein with RNA.

*Calf-spleen deoxyribonuclease.* Enzymes capable of depolymerizing DNA occur in a wide variety of cells. They are probably ubiquitous cell constituents. Only the depolymerase secreted by the pancreas (whose function is primarily digestive) has been highly purified and crystallized. The intracellular deoxyribonucleases appear to represent a functionally and chemically distinct group of enzymes, endowed with a high degree of specificity. Duplication of DNA-containing structures may be assumed to be one of the necessary steps preparatory to cell division. The mechanisms by which the cell synthesizes these macromolecules are not yet known, but the intracellular deoxyribonucleases are probably involved. Until they are isolated and characterized, however, their role in life processes cannot be established. Experiments were therefore initiated, as reported in Year Book No. 52, to develop a procedure for the isolation, purification, and crystallization of the intracellular deoxyribonuclease of calf spleen. These experiments have been continued during the past year by McDonald and Ghee. The method of purification developed thus far consists in extracting frozen calf spleen at pH 4.0, fractionating the extract at pH 7.5 with concentrations of ammonium sulfate between 0.4 and 0.75 saturation, and then preferentially denaturing inert proteins at pH 4.3 and 62° C. The final preparation contains 70 per cent of the deoxyribonuclease activity present in calf spleen; 4-5 g of protein are obtained from 10 kg of spleen. The partially purified deoxyribonuclease is still contaminated with ribonuclease.

## RADIATION STUDIES

*Effects of ionizing radiations on nucleoproteins.* During recent years we have given considerable attention to the nature of the changes induced in the living cell by irradiation. Our experimental evidence has indicated that heavy doses of X-rays do not alter the DNA in a manner that can be detected by its ability to bind either methyl green or the leucobasic fuchsin of the Feulgen reagent. Other experiments have shown, however, that irradiation can modify the stainability of cells with such dyes as pyronin and fast green, and alter the ability of cells to swell when exposed to the action of the trypsin-water-buffer-water sequence of reagents (Year Book No. 50). These changes were attributed to alterations effected by the ionizing radiations in the structural nucleoproteins.

In the light of these indications that chromosomal nucleoproteins can be altered in situ by X-rays, it seemed desirable to determine whether the pattern of organization can also be modified by irradiation after the chromosomal materials have been extracted from the cell. Inferences about biological properties derived from such studies are subject to the criticism that the materials have been altered in the course of extraction. In an effort to minimize such alteration, aqueous extracts were used, since they afford the closest approximation now known to deoxyribonucleoproteins in their native state.

Isolated cell nuclei, when placed in a small volume of water, form viscous gels; further addition of water leads, ultimately, to the formation of a solution. One of the outstanding properties of such aqueous nucleoprotein systems is their extreme instability, particularly noticeable with respect to the depolymerization of the deoxyribonucleic acid component. These facts suggested that a study of the action of ionizing radiations on aqueous nucleoprotein systems might be of assistance in resolving the paradox of the sensitivity of biological (genetic) systems to radiation

as contrasted with the insensitivity of DNA, one of the principal components of genetic materials.

Gels of nuclear materials were therefore exposed by Bernstein to low (1000 r) doses of X-rays, and viscosity measurements were made on the gels. Gel viscosity is highly anomalous, and is attributable not only to the size and shape of the constituent molecules, but also to interactions between particles, and to the resultant network-like structure. As shown in figure 10, there is

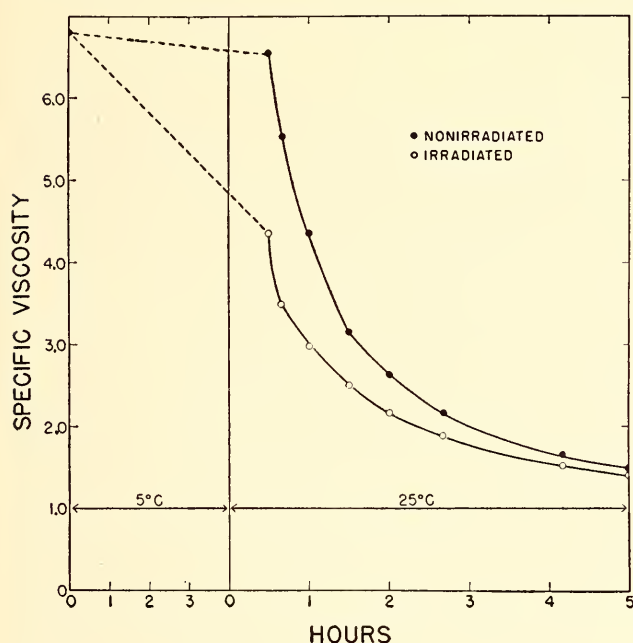


FIG. 10. Effect of X-radiation on viscosity of nucleoprotein gels. Gels contained 0.068 per cent DNA. After a dose of 1000 r, control and irradiated samples were stored at 5° C for 4 hours, then placed at 25° C and their viscosities measured at intervals.

no difference between the viscosities of irradiated and nonirradiated gels immediately after treatment. After storage for several hours at 2°–5° C, however, there is a marked decrease in the viscosity of the irradiated gel. To determine whether or not the radiation-induced loss of viscosity also involves a depolymerization of the DNA component, the viscosity of nucleoprotein gels, irradiated and stored in water, was measured either in water or after transfer to 1.0 M NaCl. In the latter case, the constituent nucleic acids and proteins are dissociated and the viscosity meas-

ured is that of the DNA. The data of table 16 show that this viscosity decrement does not relate to depolymerization of the DNA by any action of the irradiation, although there is an effect on the structural viscosity of the aqueous nuclear gel that is proportional to radiation dose.

In a further attempt to clarify the nature of the radiation sensitivity of the naturally occurring nucleoproteins, synthetic nucleoproteins were made by mixing neutral solutions of DNA and bovine serum albu-

TABLE 16  
EFFECT OF X-RAYS ON THE VISCOSITY OF NUCLEOPROTEIN GELS

| SAMPLE                                     | SPECIFIC VISCOSITY OF NUCLEOPROTEIN GELS AFTER IRRADIATION DOSES OF |       |       |        |
|--|---|-------|-------|--------|
|  | 0   | 250 r | 500 r | 1000 r |
| 0.3 mg DNA per ml;*                        |   |       |       |        |
| viscosity measured in water . . . . .      | 10.6  | 7.64  | 5.96  | 4.13   |
| 1.2 mg DNA per ml;*                        |   |       |       |        |
| viscosity measured in 1.0 M NaCl . . . . . | 2.65  | 2.80  | 2.61  | 2.54   |

\* Concentrations are expressed as amounts of DNA; the gels also contained an equal amount of protein. All samples irradiated at a concentration of 1.5 mg DNA per ml, and subsequently diluted for measurement, after storage at 2°–5° C for 4 to 6 hours.

min. As shown by viscosity measurements, these mixtures were appreciably less stable than protein-free DNA, but not so unstable as the nuclear gels.

The effects of several enzymes on the structural viscosity of the nuclear gels were examined. Of these, the proteolytic enzyme trypsin was easily the most effective in reducing the viscosity of the gels. It was also found that urea could completely destroy the structural or anomalous portion of the viscosity of the nuclear gels. These observations suggest that the radiation-sensitive structural elements of these systems are the secondary intermolecular associations between nucleoprotein molecules. The intrinsic structural instability of these molecules, which is expressed in



the form of a cross-sectional fragmentation of the deoxyribonucleoprotein molecule, appears to provide a background sensitivity, not directly involved in the radiation effects.

*Effect of ultraviolet radiation on the living cell.* During the past year an ultraviolet-sensitive vidicon tube was made available for our use by Dr. L. E. Flory of the Radio Corporation of America, and studies of living cells with ultraviolet radiation were initiated in collaboration with Dr. Moses at Brookhaven National Laboratory. Energy at 2537 Å was isolated from a low-pressure mercury arc by means of quartz prisms and used as illumination for a quartz microscope in conjunction with the television camera. Intensities required for satisfactory television images were of the order of magnitude of those used for photomicrography (about one-minute exposure on spectrographic emulsion). Preliminary studies showed that the television microscope affords a very satisfactory and efficient means of viewing an ultraviolet-microscope image for the following reasons: the image is larger, clearer, and brighter than can be obtained with a fluorescent viewer at comparable intensities; contrast may be varied for greater clarity; the image may be viewed either continuously or intermittently, and a permanent photographic record may be made from the monitor screen, if desired, with a minimum of exposure of the living material to ultraviolet radiation; the electronic magnification of the circuits provides a highly magnified image with relatively low-power optics, the resolution being limited only by the numerical aperture of the optical system. The advantages of being able to view such an image without the need of photographic processing are obvious.

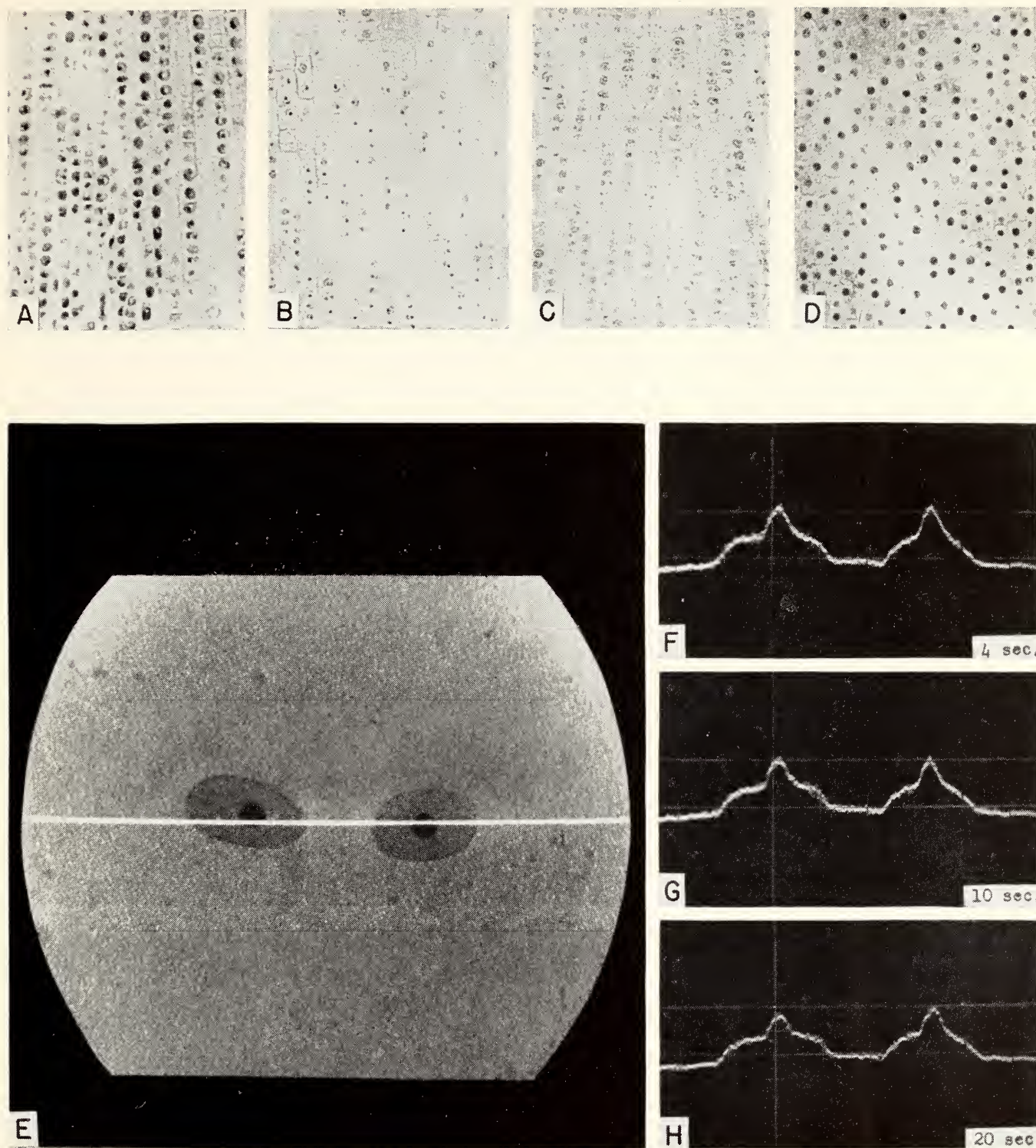
The line-selector system (described in Year Book No. 52) presents on an oscilloscope a photometric trace of any desired line scanning the television image. Thus, at 2537 Å, where absorption is due chiefly to nucleic acids, the nucleic acid concentration represented at any point in the

image of an unstained or living specimen is a function of the height of the oscilloscope trace at the corresponding point. Such photometric information is obtained more quickly and easily by this method than by photographic densitometry, but the greatest advantage of the method is that it affords, for the first time, a continuous quantitative record of nucleic acid changes in various portions of the living cell.

The application of the method is illustrated by the photographs, taken by Dr. Moses, shown in plate 1 (E-H). Two living reptilian erythrocytes are imaged on the television monitor screen. The white line is a guide line directly beneath the selected scanning line. Oscilloscope traces of the selected scanning line at 4, 10, and 20 seconds after the initial exposure are shown in figures F, G, and H. The height of the peaks due to nuclear absorption decreases 10 to 15 per cent during this time; some decrease in cytoplasmic absorption can also be distinguished. No further significant change was recorded up to 2 minutes. Reduction of nuclear and cytoplasmic absorption in cells during exposure to ultraviolet radiation is a characteristic response, but such rapid alterations as are noted above could not have been detected except by the methods employed.

An additional dramatic demonstration of absorption of ultraviolet radiation by nucleic acids in the living cell is afforded by staminate hairs of *Tradescantia* in which active cyclosis is occurring. Pronounced absorption by nucleic acids of nucleus and cytosome is apparent from the first instant of exposure of the cells to wavelength 2537 Å, while cyclosis proceeds uninterruptedly. After an exposure of 2 to 3 minutes, cyclosis is markedly retarded but is not terminated, although there occurs during this period a pronounced increase in the volume of the cell vacuole and a concomitant displacement of the cytoplasm toward the cell periphery. No marked changes in the absorption of nuclear materials occur during this period.

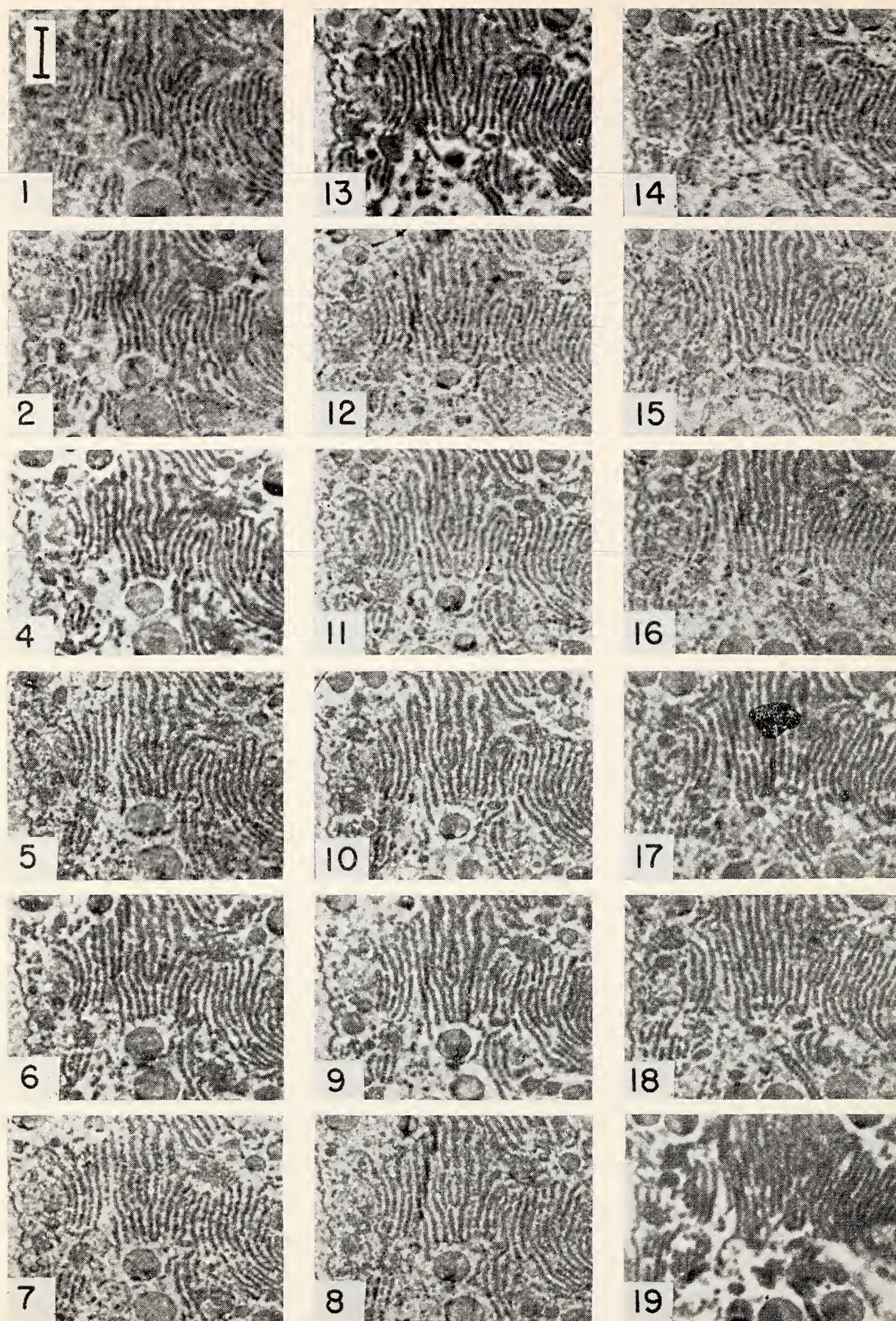




A-D. Effect of proteins on stainability of apurinic acid by leucobasic fuchsin. Paraffin sections, 7 microns thick, of onion root tips fixed in Zenker's fluid were stained with leucobasic fuchsin after immersion in 1 N hydrochloric acid for 1.5 hours at 37° C, and for 2 hours at pH 6 and 37° C in (A) distilled water, (B) 0.1 per cent aqueous solution of ribonuclease, (C) 0.1 per cent aqueous solution of cytochrome c, and (D) 0.1 per cent aqueous solution of egg albumin. (See page 247.)

E-H. Application of the television microscope for studies of nucleic acids in the living cell. E: Two living reptilian erythrocytes imaged on the television monitor screen. F, G, H: Oscilloscope traces of the selected scanning line at 4, 10, and 20 seconds after initial exposure. Fuller description in text.





Electron micrographs of eighteen serial sections through a salivary-gland cell of a larva of *Drosophila melanogaster*. The third section has been omitted because it was wrinkled. Estimated total thickness of all nineteen sections is between 1 and 2 microns. Magnification is indicated by a 1-micron bar superimposed on the first section. To the left in each micrograph is the nuclear membrane. The endoplasmic reticulum, which shows progressive changes from one section to another, is a complex structure of interconnected curved double sheets. The granules surrounded by these sheets are spherical secretion granules of the gland.



Observations of amount and rate of change in cellular constituents during the course of irradiation are of considerable interest in reaching an understanding of the reaction systems involved. Further information along these lines may be anticipated, now that the preliminary experiments have confirmed the validity of the original premise that the television microscope has enormous potentialities for quantitative spectrophotometry of living cells. Calibration and assessment of the limits of the method must first be determined, and work toward this end is being undertaken by Dr. Moses.

#### ELECTRON MICROSCOPE STUDIES

Methods of ultrathin sectioning developed during recent years have enabled the cytologist to use the high resolving power of the electron microscope for study of the fine structural details of cells. Interpretations derived from examination of electron micrographs of such cellular materials have been based for the most part on single isolated ultrathin sections. Obviously a method of securing photographs of successive sections was necessary if valid interpretations of three-dimensional patterns of cellular organization were to be formulated. This problem has been attacked by Gay. Preliminary studies were initiated in this laboratory, but the major portion of the work has been carried on at the University of Pennsylvania in collaboration with Dr. T. F. Anderson. As a result of these efforts, methods have been developed for obtaining ultrathin serial sections for electron microscopy in any quantity desired.

A series of 18 serial sections from a salivary-gland cell of *Drosophila melanogaster* is shown in plate 2. The sections, ranging in thickness from 50 to 100 m $\mu$ , were mounted on one Sjöstrand-type specimen grid. In the preparation of such material for sectioning, glands are fixed in buffered osmium tetroxide, and imbedded in *n*-butyl methacrylate. The blocks con-

taining the imbedded material are carefully trimmed under the microscope and sectioned with a glass knife, using a thermal-expansion microtome designed by Dr. Keith Porter. The sections are collected in a trough containing a 15 per cent acetone solution, whose level can be adjusted by means of an attached hypodermic syringe.

The ribbon of sections is loosened from the knife edge with a fine hair, floated onto the acetone solution, and picked up on a Formvar-coated loop (shaped like a minute table-tennis racket). Using a compound microscope, the sections on the loop are positioned over one of the slits of the grid, which is mounted on the condenser, and finally attached to the grid by elevating the condenser until its Formvar-covered surface adheres to that of the loop which carries the sections. Great precision is required in all these operations, since the space between the bars of the grid is only about 0.1 mm wide, but with patience and care ribbon after ribbon can be obtained from the same block.

Another method developed by Gay involves the imbedding and sectioning of smears of salivary-gland chromosomes. It is possible in this way to identify the regions of the chromosomes represented in a given section by comparison with a photograph of the whole smear, and to select for detailed study those loci that are of special interest because of earlier genetic and cytogenetic studies.

Alternative interpretations of submicroscopic patterns revealed by these techniques cannot be finally evaluated at this time, but even a casual inspection of plate 2 will show that the recording of such minute structural details opens up new vistas for analysis of the gene and of nuclear-cytoplasmic relations. By way of illustration, three-dimensional models reconstructed from serial sections of salivary-gland cells similar to those shown in plate 2 have demonstrated that differentiated regions of chromosomes (probably heterochromatic)



are in intimate contact with the nuclear membrane, which in this region protrudes as a flasklike extension into the cytoplasm.

This structural organization appears to offer a mechanism for transfer of chromosomal products to the cytoplasm.

## MUTATIONS IN MAIZE AND CHROMOSOMAL ABERRATIONS IN NEUROSPORA

BARBARA McCLINTOCK

During the past year, studies of mutation-controlling systems in maize were continued at Cold Spring Harbor, and examinations of chromosomal aberrations in *Neurospora* were carried out at the California Institute of Technology.

### MUTATIONS IN MAIZE

In previous Year Books, evidence has been presented concerning the nature of action of mutation-controlling systems in maize that are responsible for the behavior of mutable genes. Much of the detailed evidence was derived from study over a period of years of the system composed of the two chromosomal units, *Ds* and *Ac*. Extensive examination of other systems was postponed until the behavior of this system had been well explored. Knowledge of gene-controlling systems in maize is progressing rapidly through the efforts of investigators at other institutions who are examining some of the gene-controlling units that have appeared in their materials. One such unit, being investigated by Dr. R. A. Brink and his students at the University of Wisconsin, behaves much like *Ac*. My studies of the *Ds-Ac* system during the past year have been limited to obtaining some additional information about its action at one selected locus in the chromosome complement. This has involved further examination of the seven cases of change in action of *I* induced by *Ds* when the latter was located just to the left of *Sh*<sub>1</sub> in chromosome 9. The origins and general descriptions of these cases were outlined on pages 231-233 of Year Book No. 52. The newly acquired information provides a clearer understanding of the similarities and differences among them. Only a sum-

mary of this information need be presented here.

Among the seven cases in which the action of *I* (located approximately four cross-over units to the left of *Ds* in the parent plants) was modified, *Ds* has been found to be present in the chromosome in each case and apparently not changed in location by the event that altered genic action in the segment of chromatin to the left of it. *Ds* remains near, and to the left of, *Sh*<sub>1</sub>. The seven cases may be separated into two main classes: four that regularly produce viable homozygotes, and three that have given no viable homozygotes even though many attempts have been made this past year to obtain them. In all seven cases, the chromosome 9 having the altered region that includes the locus of *I* is transmitted normally through the female gametes; but transmission through the pollen grains is considerably reduced when these compete with others carrying normal chromosomes 9. The extent of this reduction is approximately the same for all the members of the first class and for two of the members of the second class, but is extreme for the remaining member of the second class. The seedlings derived from the homozygotes produced by members of the first class have all shown the same type of albescence phenomenon—disappearance of chlorophyll from the seedling leaves at about the three-leaf stage. Intercrossing has produced individuals carrying all possible combinations of the seven altered chromosomes 9, except combinations between those of class 2, which are apparently as inviable as are the homozygotes in each member of this class. Kernels with viable combinations show no pigment in the aleurone layer, resembling in this respect

the phenotypes of the homozygotes of class 1. The seedlings derived from them exhibit the same type of albescence phenomenon that characterizes the seedlings of the class-1 homozygotes. In none of the seven cases has there been any evidence of crossing over within the affected region in individuals having a normal and an altered chromosome 9. In each case, however, crossing over to the right of the affected region—between *Sh*<sub>1</sub> and *Bz* and between *Bz* and *Wx*—is normal.

The above-described relations among the seven independent cases of change in genic action within a segment of chromosome 9 located to the left of *Ds* are consistent with a hypothesis that they originated through removal of a similar segment of chromosome 9 in each case. The common segment removed would include the region beginning just to the left of the locus of *Sh*<sub>1</sub> and ending somewhere beyond that of *I*. The differences between members of the two classes and among members of the second class could then be explained by differences in the extent of loss beyond the locus of *I*—the members of class 2 having longer deficiencies than those of class 1, and one member of class 2 the longest. No direct evidence of deficiency in any one of these seven chromosomes was obtained, however, when each was compared with a normal chromosome 9 at the pachytene stage of meiosis. Also, it is known that deficiency need not be involved in such loss of genic action. A *Ds*-induced inhibition of action may be the cause, for this is known to account for some similar types of loss of genic action in the segment of chromosome 9 located to the right of *Ds*. The types and extents of such loss were described in Year Book No. 52.

*System controlling genic expression of  $a_1^{m-1}$ .* The occurrence of genic instability in many organisms, and its general resemblance to cases in maize, suggest that the gene-controlling units found in maize are not peculiar to that species. Rather, they reflect a type of nuclear organization and action that is probably present in many

organisms. Thus it is necessary, before wider integrations can be made, to determine the kinds of gene-controlling systems that exist, how they differ from one another, and whether or not interactions occur among them. Attention is now being focused, therefore, on a system controlling genic expression which differs from the *Ds-Ac* system in some respects but resembles it in others. Although knowledge of the behavior of this second system is limited, it has been possible to formulate an interpretation from present information. This system operates to control genic expression of  $a_1^{m-1}$ , one of the mutable conditions that has arisen at the locus of *A*<sub>1</sub> in chromosome 3 in the Cold Spring Harbor cultures. (*A*<sub>1</sub> is associated with the appearance of anthocyanin pigmentation in plant tissues and in the aleurone layer of the kernel;  $a_1$ , known recessive allele, when homozygous, results in absence of anthocyanin pigmentation in both plant and aleurone.)

The system controlling genic expression at  $a_1^{m-1}$  is considered to be composed of two units, one inserted at the locus of *A*<sub>1</sub> in chromosome 3 at the time of origin of  $a_1^{m-1}$ , and one located elsewhere in the complement. The evidence suggests that this latter unit, tentatively designated as *Spm* for reasons that will be made evident shortly, may be altered in somatic cells and possibly may also be transposed from one location to another in the chromosome complement. In these respects, its behavior resembles that of *Ac*. Another apparent similarity is that *Spm* must be present in the nucleus if mutations are to occur at the locus of  $a_1^{m-1}$ . *Spm* and *Ac* differ in a significant way, however, and this is related to the contrasts in phenotype that are expressed in their presence and absence. The presence of *Ac* is detected by the alterations it induces at the locus of the gene whose mutations it controls (or at the locus of *Ds*), which lead to recognizable phenotypic modifications in somatic cells; its absence is indicated by the absence of any such modifications. With



regard to *Spm*, however, its presence or absence is recognized not only by the appearance or nonappearance of mutations at  $a_1^{m-1}$ , but also by another phenotypic difference that is sharply expressed in plants having certain states of  $a_1^{m-1}$ . When *Spm* is present in somatic nuclei, genic action at the locus of  $a_1^{m-1}$  is completely inhibited except in those cells that carry a mutation at this locus. Such mutations occur in some somatic cells during development. They permit the occurrence of some forms of action of the genic material at the locus of  $a_1^{m-1}$ , and this is recognized phenotypically in the tissues produced by the descendants of such cells. Therefore, both plants and kernels that have *Spm* are variegated in anthocyanin pigmentation. Spots or areas, each exhibiting a particular type and intensity of pigmentation—for different types of mutations are induced at  $a_1^{m-1}$  when *Spm* is present—appear on a colorless background. Removal of *Spm*, whether occurring somatically in some cells or produced as the consequence of meiotic segregations in plants heterozygous for it, results in partial release of inhibition of genic action, presumably at  $a_1^{m-1}$ , for the tissues of both the plant and the aleurone layer of the kernel are uniformly pigmented. With some of the states of  $a_1^{m-1}$ , the color is less intense than that which appears when the normal  $A_1$  locus is present. The difference may be qualitative as well as quantitative, for the pigment does not seem to be the same as that produced by  $A_1$ . No spots or areas with altered pigmentation appear. In other words, no variegation is expressed in the absence of *Spm*. The symbol *Spm* has been used to designate this unit because it acts both as a suppressor and as a mutator.

The origin of  $a_1^{m-1}$  from a modification occurring at the locus of the normal  $A_1$  gene was described in Year Book No. 50 (1950-1951). *Spm* was first discovered in a study of a particular state of  $a_1^{m-1}$ , derived from the original state. This derived state was selected early in the investigation of  $a_1^{m-1}$  because it was considered to be

more suitable than the original state for an analysis of factors responsible for mutation at  $a_1^{m-1}$ . It was present in an individual kernel in the progeny of the plant in which  $a_1^{m-1}$  first appeared. Some aspects of the inheritance behavior of this state of  $a_1^{m-1}$  were outlined in Year Book No. 52. The evidence described there indicated the presence in variegated plants of a unit factor (or of several similar unit factors), not linked to the locus of  $a_1^{m-1}$ , that influenced the expression of  $a_1^{m-1}$ . It was then thought that removal of this factor from nuclei by meiotic segregations resulted in a particular type of mutation at the locus of  $a_1^{m-1}$ , and that in this respect the case resembled that of  $a_1^{m-2}$ , where removal of such a factor is known to lead to mutation at  $a_1^{m-2}$ . Results of subsequent investigation, however, carried out during the past year, suggest that removal of the unit factor, now designated *Spm*, does not lead to mutation at  $a_1^{m-1}$ , but rather removes an inhibitory action ascribable to *Spm*, as outlined above.

Evidence suggesting linkage of an *Spm* factor with *Y* or its allele *y* (*Y*, yellow starch in endosperm, dominant to *y*, white starch), located in chromosome 6 of the complement, was presented in Year Book No. 52 in table 3 of the report of this Department. An exploratory test involving only nine plants was made this year, partly in order to determine whether or not the apparent linkage could be validated. These plants were derived from the variegated kernels produced on the ear of plant 6046C-2, recorded in the above-mentioned table. Two or more independently located *Spm* units were assumed to be present in this plant, but one of them appeared to be linked to *Y*. It should be emphasized that, in backcross tests, segregation ratios typical of linked units would appear only on the ears of plants having a single *Spm* unit, and in such plants only when losses of *Spm* from many cells did not occur late in the development of the ear. Furthermore, transpositions of *Spm* to new locations would distort ratios or even eliminate

evidence of linkage if they occurred early in the development of an ear or if they were frequent during the later stages of development. An early-occurring loss of *Spm* from a cell whose descendants contribute to the formation of the ear is detected readily by the appearance of an area on the mature ear in which all the kernels carrying  $a_1^{m-1}$  show the characteristic pale

cated approximately 38 crossover units from Y. The presumed location in chromosome 6 of one of the *Spm* units in the parent plant, 6046C-2, has thus been confirmed.

Further description of the behavior of this gene-controlling system will be postponed until the evidence from tests now under way becomes available.

TABLE 17

PHENOTYPES OF KERNELS APPEARING ON EARS OF PLANTS WHOSE CONSTITUTIONS WERE  $a_1^{m-1} Sh_2/a_1 sh_2; Y/y$  WHEN THESE WERE CROSSED BY PLANTS HOMOZYGOUS FOR  $a_1, sh_2$ , AND  $y$

| PARENTAGE<br>IN CROSS |           | PHENOTYPES OF KERNELS  |     |            |     |           |   |                        |   |            |   |           |     | Totals |
|-----------------------|-----------|------------------------|-----|------------|-----|-----------|---|------------------------|---|------------|---|-----------|-----|--------|
|                       |           | <i>Sh</i> <sub>2</sub> |     |            |     |           |   | <i>sh</i> <sub>2</sub> |   |            |   |           |     |        |
|                       |           | Color in aleurone      |     |            |     |           |   | Color in aleurone      |   |            |   |           |     |        |
|                       |           | Pale                   |     | Variegated |     | Colorless |   | Pale                   |   | Variegated |   | Colorless |     |        |
| ♀                     | ♂         | Y                      | y   | Y          | y   | Y         | y | Y                      | y | Y          | y | Y         | y   |        |
| 6629A-1               | 1041-5... | 36                     | 58  | 66         | 35  | 0         | 0 | 0                      | 0 | 0          | 0 | 85        | 89  | 369    |
| 6629A-1               | 1041-4... | 33                     | 54  | 51         | 36  | 0         | 1 | 0                      | 0 | 1          | 1 | 101       | 98  | 376    |
| 6629A-3               | 1040-1... | 34                     | 52  | 43         | 37  | 0         | 0 | 0                      | 1 | 1          | 0 | 85        | 83  | 336    |
| 6629A-4               | 1041-5... | 23                     | 65  | 56         | 36  | 0         | 0 | 0                      | 0 | 0          | 0 | 90        | 84  | 354    |
| 6629A-6               | 1040-1... | 34                     | 67  | 78         | 37  | 0         | 0 | 0                      | 0 | 1          | 0 | 86        | 113 | 416    |
| 6629A-7               | 1040-1... | 29                     | 59  | 58         | 36  | 4         | 1 | 0                      | 0 | 0          | 0 | 105       | 100 | 392    |
| 6629A-9               | 1040-8... | 39                     | 41  | 49         | 38  | 0         | 0 | 1                      | 0 | 0          | 0 | 79        | 90  | 337    |
| 6629A-9               | 1040-5... | 41                     | 58  | 50         | 34  | 0         | 1 | 0                      | 1 | 0          | 0 | 79        | 115 | 379    |
| Totals                | .....     | 269                    | 454 | 451        | 289 | 4         | 3 | 1                      | 2 | 3          | 1 | 710       | 772 | 2959   |

aleurone color that develops in the absence of *Spm*. A few ears had such areas. Early-occurring transpositions of *Spm* can be detected by markedly altered linkage relations between the given units, by absence of such linkage, or by linkage of *Spm* to a factor carried in another chromosome. There was evidence of such changes in location of *Spm*, based on observed ratios produced by some of the ears.

The conditions, indicated above, that are required for the production of typical linkage ratios in backcross tests were present in eight ears obtained from six of the nine tested plants. The design of the test cross and the types of kernels that appeared on each of these eight ears are shown in table 17. From the data in this table it may be concluded that a single *Spm* unit was present in these six plants, linked to Y and lo-

#### CHROMOSOME ABERRATIONS IN NEUROSPORA

During the winter of 1953-1954, examination of chromosome complements in asci of *Neurospora crassa* was undertaken at the California Institute of Technology, for the purpose of determining the nature of chromosomal aberrations known to be present in strains 4637 and 45502. All the stocks used in this study were obtained from Mary B. Mitchell, and all crosses were made by her. Her co-operation and interest were very much appreciated.

Previous investigations had shown that strain 4637 carries a reciprocal translocation between two nonhomologous chromosomes. Although the earlier studies were limited, they did indicate that chromosomes 1 and 6 are involved in the translocation. The purpose of this year's study was



to determine the position of break, in each of these two chromosomes, that gave rise to the translocation. Crosses were made between the wild-type strain 2522-1A and strain 4637R-1A. Nuclei in asci produced by this cross were examined at that stage of the first meiotic prophase when the chromosomes are maximally elongated. It was possible, by observing synaptic configurations of the chromosomes involved in this translocation and by comparing chromomere morphologies of paired elements within it, to determine the break position in each of the chromosomes.

The linear organization of chromosome 6, the next to the smallest chromosome of the complement, is unusually well defined, and this makes possible a ready identification of its component parts. That of chromosome 1, the longest chromosome of the complement, is less well defined; but two conspicuous chromomeres are present, which can serve as points of orientation in describing break positions. One of them is located near the middle of the chromosome, dividing it into two segments with relative lengths of 1 and 1.6. The other conspicuous chromomere is located near the end of the shorter of these two segments. The position of the break in chromosome 1 is in the longer of its two segments, approximately three-eighths of the distance from the free end of the segment. Chromosome 6 may be divided into several well marked segments. One terminal segment contains large, closely packed chromomeres. This is followed by a longer segment with smaller, less closely aligned chromomeres. A very conspicuous, deep-staining, dumbbell-shaped chromomere separates the second segment from the remaining segment of the chromosome. This last segment, whose length is about one-quarter that of the total chromosome, contains a few small, rather widely separated chromomeres. The position of the break in chromosome 6 is in the second of the above-described segments, closer to the first segment than to the conspicuous dumbbell-shaped chromomere.

In crosses of strain 4637 to wild type, various types of synaptic configuration were produced by association of the two translocation chromosomes introduced by strain 4637 and the two corresponding normal chromosomes introduced by the wild-type strain. Homologous association of all components was frequent, resulting in the formation of a cross-shaped configuration having three short arms and one long arm. Failure of association of homologous components of one or more of the shorter arms of the cross was observed, however, in a number of nuclei. The remaining five pairs of chromosomes appeared to be normal, for no gross structural modifications were observed in any member of a pair.

In contrast to the relative ease with which the structural modification present in strain 4637 could be analyzed, that associated with strain 45502 proved to be very difficult. In crosses of this strain to wild type, a distinctive pattern of normal and abnormal spores appeared in many of the asci. Cytological examination indicated that there is no simple reciprocal translocation between two nonhomologous chromosomes in this strain to account for the distinctive patterns of spore types. The chromosome complements of four isolates of strain 45502 were examined: T45502-P1315-1A, T45502-1507-1A, T45502-1508-1A, and 70007TR-2A. The examinations suggested that in each of them the complement is composed of seven haploid chromosomes plus an extra chromosome whose length is approximately half that of the smallest chromosome of the complement, namely, chromosome 7. The origin of this extra chromosome could not be determined readily by inferences from synaptic associations, because of the many irregularities in chromosome associations that occurred when these strains were crossed to wild type and even when two of them, T45502-P1315-1A and 70007TR-2A, were intercrossed.

A brief description of some of the abnormal synaptic associations that were observed at the first meiotic prophase in asci

produced by crossing any one of the four strains of 45502 with wild type will indicate the nature of the complexities encountered in this analysis. One end of the fragment chromosome was often associated with an end of another chromosome. This did not necessarily reflect homology, because any one of the seven chromosomes of the haploid complement could enter into such an association. It occurred most frequently, however, with chromosome 7. When one end of the fragment chromosome was associated with an end of another chromosome, the homologue of this second chromosome sometimes formed a terminal association with a member of still another pair of chromosomes. Although most of the aberrant associations occurred between ends of chromosomes, a few nuclei were observed in which longer segments of two nonhomologous chromosomes were synaptically associated.

Because of these irregularities in synaptic behavior, determination of chromosome composition and organization in the ascus nuclei was difficult. In all nuclei, pairing occurred between some of the homologues. The same pairs, however, were not present in each nucleus. Nevertheless, by noting in each nucleus which chromosomes were paired and by comparing the members of each pair with regard to their internal structure, it was possible to determine whether or not any structural rearrangement was present in any member of a given pair. For two of the four examined isolates of 45502, this method produced no evidence of gross structural modifications in any of the seven regular chromosomes of the complement. In the other two isolates, the composition of chromosome 7 was not determined with certainty, but the other six chromosomes appeared to be normal.

As was stated earlier, the origin of the small extra chromosome present in each of the four isolates of 45502 was not determined. The high frequency of association of this fragment with a member of the chromosome 7 pair suggests a possible deri-

vation from chromosome 7. Some support for this inference is given by the similar high frequencies of such associations that appeared in many of the nuclei produced by crossing 45502-P1315-1a with 70007TR-2A. Each parent presumably contributed the seven haploid chromosomes plus the fragment. Thus it was to be expected that nuclei with eight homologously associated pairs of chromosomes would be numerous. Instead, they were relatively rare. More often, one or both of the fragment chromosomes were synaptically associated with another chromosome of the complement. Sometimes there appeared very complex configurations, involving members of several different chromosomes of the complement. Chromosome 7 was a component of most of these aberrant configurations.

The aberrant synaptic associations appearing at the first meiotic prophase in all crosses involving these four isolates of 45502 were reflected at the late diakinesis and metaphase stages. Associations between nonhomologous chromosomes were noted. Also, univalents were present in a number of nuclei. No concerted effort was made, however, to analyze these stages, or still later ones in the meiotic process. Therefore it is not known whether or not many abnormal disjunctions of chromosomes occurred at anaphase I as a consequence of abnormal pairing or lack of pairing. Neither is it known to what extent such disjunctions could contribute to abnormal spore formation.

The difficulties encountered in the analysis of the chromosome constitution of 45502 were intensified in the first period of observation by the presence of another chromosomal abnormality, which was later found to have been introduced by the wild-type parent. The initial observations were made in asci produced by the cross of 45502-P1315-1a to wild-type strain 2292-2A. In addition to the fragment chromosome, a structural abnormality was noted in one of the two chromosomes 5. This chromosome was considerably longer than its normal homologue, its length being com-



parable to that of chromosome 3. A segment of uncertain origin, added to or inserted into one arm of the chromosome, was responsible for the increased length. Synaptic associations were very frequent between this abnormal segment and the fragment chromosome. In addition, other complex types of synaptic configurations were formed, incorporating not only the fragment chromosome and the abnormal chromosome 5, but also one or more other chromosomes of the complement. Frequently, homologues of chromosome 7 or of both chromosome 7 and chromosome 2 were components of these configurations. Only after an analysis had been made of the chromosomes in asci derived from other crosses was it realized that the abnormal chromosome 5 had been introduced by the wild-type parent 2292-2A.

Confusion in interpreting the chromosome constitution of 45502—arising from this initial lack of knowledge of the constitution of the wild-type parent, 2292-2A, used in some of the crosses—seriously ham-

pered progress toward a solution of the main problem, which was to determine the chromosome abnormality responsible for the distinctive spore pattern and for the accompanying false linkages of certain genetic markers. Nevertheless, the knowledge gained from this experience is of some general significance. It indicates the necessity for determining the chromosome constitution of wild-type and tester stocks before they are used in crosses requiring cytogenetic analysis. It also suggests that some of the discordant results derived from genetic analyses may be due to undetected structural modifications of chromosomes in strains presumed to be normal in their chromosome constitutions.

Although the study of chromosome constitutions and behavior in strains derived from 45502 had to be terminated before the cytogenetic relations had been clarified, the increased knowledge of chromosome organization and behavior in *Neurospora* which it provided will serve as a guide in future studies of this type.

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## DEPARTMENT OF ARCHAEOLOGY

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Field work, which is principally the gathering of raw data, in archaeology is both a fascinating and a worrisome task. The removal of any shovelful of earth, the turning of a seemingly casual stone may lead one into work involving considerable time and effort, the significance of which can often be assessed only after the work is completed. The mass of material available to the excavator of any sizable archaeological site is so great that he cannot hope to handle all of it. He is constantly faced with decisions of what to exploit, what to by-pass; and he must weigh the whole effort at any site or in any region against other work in the larger area of his researches. Not only must he thus select with care the locations of his excavations, regretfully turning his back on situations that he would like to explore further or that hold tantalizing promise of worth-while results, but he also must look forward to the less exciting task of painstakingly labeling and recording the numerous small objects and the wealth of larger remains that emerge as the products of his digging. It is at this stage of his work that the archaeologist, half smothered under the weight of his accumulations, strives hard to see the forest from the trees and sometimes wonders if his methods are not open to improvement.

With these thoughts in mind it is heartening to be able to report that the results of the past field season, which involved sizable excavations at the large ruins of Mayapan, and survey combined with lesser digging elsewhere in the peninsula of Yucatan, have been most satisfying. Discoveries that first appeared as isolated phenomena are falling into place and forming a pattern that portrays interesting aspects of the life and times of the last important era of the prehistoric Maya, the subject of

our present program of research. These findings range all the way from discoveries that throw light on the religious and aesthetic concepts of the people to the more conventional archaeological reconstructions concerning the distribution of cultures and phases of culture in time and space.

Early in the course of our work at Mayapan it was realized that one of the outstanding opportunities offered at the site was a study of the remains of houses, for the most part presumably the dwellings of ordinary people, a side of Maya archaeology that had been sadly neglected. The surface survey and numbering of all structures within and adjacent to the great wall of the city has been completed. A. L. Smith and Ruppert report that a total of 4140 buildings and platforms have been recorded. Approximately 2100 of these appear quite surely to have served as homes, a figure that might well be increased were we able at this time to identify the function of certain questionable types of remains. Aside from the steadily accumulating information concerning burial, household religion, and the simpler sides of the culture of the time, some aspects of which have already been reported, the material provided by this survey, which will be amplified by additional excavation, offers the basis for the most accurate estimate of population that has yet been made of any ancient Maya city. It would be premature to mention any figure at this time, but the simple count of houses suggests that the settlement was large in terms of present-day Yucatan.

The large-scale plan of the ceremonial center at Mayapan, a project begun last year to provide the basis for comparative architectural studies, was completed this season. In an area approximately 200 by 300 m there are no less than 114 structures



varying in size from the great pyramid-temple, the so-called Castillo, to small shrines, dwellings, and simple platforms. Proskouriakoff, who performed the mapping and has carried on preliminary studies of the architecture, notes that this is a far more crowded assemblage than is found at earlier ceremonial centers, and suggests that it came about, at least in part, from a decentralized ritual organization serving a multiplicity of cults.

As the mapping and architectural studies have progressed, there has been further definition of types of structures and of function. The dominant type of civic or religious building is the colonnaded hall. An interesting aspect of these buildings, which, because of their locations and certain internal features, have mostly been thought of as being for ceremonial use, is that some may have had a residential function. This is indicated by the close association of dwelling-type structures that seem to have been for the purpose of providing household services for the halls. Proskouriakoff raises the question whether the Mayapan hall is not the counterpart of the ancient Mexican "Telpuchcalli," the bachelors' or men's house, or the "Cal-mecac," a monastery or place for the training of novitiates, buildings that are described by the sixteenth-century writer Sahagun as having existed at Tenochtitlan, the Aztec capital.

Major excavations, as in the previous field season, centered in and around the ceremonial center at Mayapan. Although most of this work was directed toward the study of the civic and religious architecture, a phase of our explorations that is now virtually completed, some attention was given to the better class of residences, presumably the palaces or houses of the aristocracy. Fourteen temples, colonnaded halls, shrines, and platforms were explored by Shook; by Winters, a graduate student in anthropology at the University of Chicago, working with this Department; by P. E. Smith and Irving, graduate students in anthropology at Harvard University,

also working with the Department; and by Pollock. Lesser excavations in ceremonial buildings were carried out by Proskouriakoff, Pollock, Strömsvik, and R. E. Smith. Proskouriakoff, Irving, and Thompson tested and explored several imposing residential groups and structures. Detailed accounts of this work will be found in succeeding sections of this report. A few of the more interesting discoveries and some of the broader implications of our findings will be mentioned here.

The finding by Winters of a group of effigy incense burners of pottery, badly broken but with most of the pieces present, enabled him to restore a number of those elaborate vessels. Although sherds of this type of censer are found in tremendous quantities, the pottery is so friable that a vessel normally disintegrates into hundreds of pieces; up to this year it had not been possible to restore a single example to anything approaching completeness. The censers, in which copal, the native incense, was burned, are typically large, some 40 or 50 cm high, with an anthropomorphic figure, modeled usually in the full round, applied to the side of the vessel. The figures are of gods or of men impersonating gods. They are elaborately painted and show in detail attributes of the deities or symbols of office, ceremonial objects, weapons, and accouterments of dress. Guided by the restored vessels, we should be able to identify fragments that previously were without meaning and to restore considerable sections of other censers. A study of the details of the figures offers promise in a subject that is otherwise scantily represented by archaeological remains.

Many of our excavations at Mayapan have produced fragments of wall plaster that show traces of painting. These occur with sufficient frequency to indicate that architectural decoration of this sort was common. It is rare, however, to find any sizable area of plaster still adhering to a wall, and no painted designs, other than those on fragments so small as to give little idea of the original, had been found prior

to this year. During the past season Winters' excavations in a temple uncovered the wall of a room some 5 m long that retained fairly large areas of painting on the plastered surface. Most of the paint was on the lower half of the wall, only vestiges of the scene remaining on the upper part. As the mural had been covered by a later coat of plaster and much of the underlying plaster had pulled away from the wall, ready to crumble and fall at any moment, the removal of the surface layer and cleaning of the painting presented no small difficulty. This task was undertaken by Winters and a native assistant, and was carried on while the mural was being copied.

Under these difficult conditions, complicated by dust and sun, Proskouriakoff made a watercolor reproduction of the painting. The key to understanding the subject portrayed rested largely in deciphering, and relating to the rest of the mural, the fragmentary details on the upper part of the wall. Proskouriakoff's solution of the problem and restoration of the composition were brilliantly successful. The scene depicts five buildings, possibly temples, resting on a low platform. The walls of the buildings and platform are elaborately decorated with painted designs. The architectural style suggests a mixture of Maya and Mexican traditions and is of a sort not suspected from the remains of buildings at Mayapan or elsewhere in Yucatan.

In our last annual report it was noted that the late stage of Maya history represented by the principal remains in Mayapan seemed to have witnessed some rather profound changes in general cultural level and in religious structure as compared with the old classic Maya civilization. Aside from the obviously inferior art and architecture, this change was expressed in the poverty of ceremonial offerings. The numerous shrines, apparently a late development, suggested the decline in importance of a centralized religious organization and the growth of a multiplicity of

cults. This process of decentralization may have attained its ultimate expression in the transference of much of what remained of religious ceremonialism to the private dwelling.

Our excavations and explorations this season in the finer residences are revealing in respect to these matters. The shrine room in the house or the separate shrine in the courtyard before the house appears to be customary, if we may judge by the examples so far explored. More surprising is the fact that both in excellence of building and in quality and variety of ceremonial objects the important houses thus far tested have proved richer than the temples and other ceremonial structures. One is tempted to picture a time when the gods were slighted in favor of personal comfort and glory. More evidence is needed, but in an era of decadence, which is clearly indicated by inferior art forms and the falling-off of craftsmanship as compared with earlier times, it is not surprising to find cultural decay and materialism walking hand in hand.

In the native literature and early Spanish accounts that deal with the preconquest history of Yucatan there are numerous references to the fall of Mayapan, the last great city of the Maya. This event, which presumably occurred about A.D. 1450, is variously referred to as the "abandonment," "depopulation," or "destruction" of the town, and is often associated with fighting of the nature of civil war. Our excavations in 1953 uncovered several instances of the apparent looting of altar deposits, and it seemed clear that this had been done in ancient times, before the buildings that housed the altars had collapsed. Although these findings suggested insurrection or invasion and pillage, that interpretation was by no means certain. The gradual abandonment of the city from causes other than strife, and the breakdown of old social and religious forms, might in time leave the empty temples as open to acts of vandalism as the more cataclysmic effects of war.



Discoveries of the past season have thrown light on the nature of the abandonment of Mayapan. Not only have we uncovered several more examples of the robbing of altars prior to the collapse of the buildings, but we have noted three instances of charred timbers beneath the debris covering the floors of structures. These charred timbers are unquestionably the remains of roof beams. As it would be relatively difficult to set fire to the wooden supports in a beam-and-mortar roof, owing to the nature of the construction, there is good evidence that the buildings were purposely burned. There is also evidence from the pottery on the floors that this occurred late in the occupation of the city and that, in at least one instance, the building was in use at the time of destruction (see p. 280). These findings clearly suggest that the sack and destruction of the city by hostile forces, as implied by old records, did in fact take place.

In our annual report of two years ago (Year Book No. 51, pp. 235-241) we discussed a number of problems dealing with the history of Yucatan during the five centuries preceding the Spanish Conquest. The gist of those remarks was that a reappraisal of the archaeological evidence might lead one to question prevalent reconstructions of the history of that period. The work of Ruppert, Shook, A. L. Smith, and R. E. Smith at Chichen Itza this season has provided additional information bearing on these questions. The survey of house mounds there discloses no dwellings like those at Mayapan; the collections of stratigraphic samples of pottery show a very small proportion of types assignable to the Mayapan Period. This leads the investigators to suggest that the permanent occupation of Chichen Itza terminated at or near the end of the Toltec Period at that city, otherwise known as the Period of Foreign Domination. They further suggest that the small amount of ceramic remains of the Mayapan Period, or Period of Maya Resurgence, represents no more than the leavings of pilgrims who jour-

neyed to their holy well, the Sacred Cenote, to make sacrifice. This, in the main, is confirmation of one of the standard reconstructions of Yucatan history.

Whatever the final answer to these questions—and the evidence is not yet conclusive—a matter of equal importance is that the ceramics of Chichen Itza and Mayapan strongly indicate that the major break or change in cultural tradition occurs not at the beginning of, or during, the Period of Foreign Domination, but at the close of that period and with the ascendancy of Mayapan. This revives questions raised earlier as to the character of the later period and the correctness of our designation of those times as the Period of Maya Resurgence, questions discussed in the annual report referred to above.

The foregoing paragraphs have touched on some of the wider implications of our findings of the past season. Detailed reports on this work will be found in succeeding sections. There also will be found reports on explorations in the vicinity of Mayapan and on solidification of buildings at Mayapan by Strömsvik, on the ceramic stratigraphy of Mayapan and its vicinity by R. E. Smith, on the art and artifacts of Mayapan by Proskouriakoff, on explorations in Quintana Roo by Strömsvik, Pollock, and Berlin, on archaeological reconnaissance along the east coast of Yucatan by William T. Sanders, and in Tabasco and Campeche by Heinrich Berlin, Carnegie Institution Fellows with this Department, on research in ceramic technology by Shepard, and on field studies of Maya agriculture by Joseph A. Hester, Jr., also a Carnegie Institution Fellow with this Department.

A project outside our Middle American program is the work of Morris in the southwestern United States. Most of the period under review was spent on the preparation of two manuscripts. One is a study of Anasazi cloth sandals. This type of footgear was made and used by Pueblo peoples of the northern Southwest for a thousand years. In the great number

of representative specimens which have been recovered from the dry caves of northeastern Arizona there lies a record of perhaps the greatest aesthetic and technological achievement of the pre-Spanish Pueblos. It is the purpose of the study to present in some detail the life history of this sandal-making art.

The second manuscript is a report on Basket Maker III sites near Durango, Colorado. It is a sequel to the report on Basket Maker II sites in the same region which is now in press. The second report will complete the record of excavations conducted by the Institution in 1938, 1939, and 1940 in the vicinity of Durango.

For his outstanding contributions to Southwestern and to Middle American archaeology, Morris received the Alfred Vincent Kidder Award of the American Anthropological Association for 1953.

#### MAYAPAN, YUCATAN

A. L. SMITH AND KARL RUPPERT

About two-thirds of the 1954 field season, January 16 to May 1, was spent in completing the square-by-square surface examination of the structures within the Great Wall of Mayapan or directly associated with the site. This survey, started in 1951, had occupied most of Ruppert's and Smith's time during the three preceding field seasons (see Year Book No. 50, pp. 230-232; No. 51, pp. 243-244; No. 52, pp. 256-258). The remainder of the 1954 season was devoted to excavation at Mayapan and exploration at Chichen Itza. Ruppert also investigated several small sites in the environs of Telchaquillo.

The survey of Squares Y, Z, and AA this year completed the surface examination (see Jones, 1952, C.I.W., Current Report 1, map). During the season 813 structures were inspected, making a total of 4140 examined and recorded on file cards since the beginning of the survey. This is naturally not the exact total number of structures at the site, for not only were there probably some that have been destroyed

completely in the building of the many modern walls, but further excavation may bring other structures to light. The approximate number of buildings which from their remains can be said to have served as homes is 2100. There are, however, many platforms with no traces of superstructures that may well have supported buildings, constructed entirely of perishable materials, which possibly served as dwellings. These are often found associated with house groups and may, of course, have been kitchens or have had some other function. Altar shrines also are often associated with family groups. Although no new types of dwelling were found this season, some refinements or amendments were noted in the general categories established during previous years.

As has been the custom in past seasons, the presence of Puuc-type stones in buildings was noted, and the location of metates was indicated. Almost all metates are of the legless, grooved kind. Several fragments of metates with legs were found, however, and there was one complete example, including the mano. The metates without legs are of limestone; the kind with legs are of tufa, a material not indigenous to Yucatan. An unusual specimen recovered is one with legs and grooved.

During the first part of the season several days were spent in a small group of mounds in Square A, about 250 m outside the city wall. The group, which rests on a slight rise, is surrounded by a stone wall averaging about 1 m high and 1.4 m thick with an entrance in its north end. Several pits were sunk in one of the structures, exposing a tomb containing two skeletons. The pottery recovered all proved to be of the Mayapan Period.

Structure Q-62 was chosen for complete excavation, as it was situated in the main ceremonial group and was a good example of an average dwelling. It was also hoped that its relationship in time could be established with a near-by colonnaded hall,



Structure Q-64. Unfortunately this could not be done. The dwelling consists of a front area with three benches and a back part divided into three rooms, the central room having a bench along its back wall. Passageways lead from the front to the rooms in back. The west rear room has a doorway opening out of its west side. The lower portion of the walls was made of stone and probably never rose more than 50 cm. The upper part of the walls and the roof undoubtedly were of perishable materials. Two burials were found associated with Structure Q-62. One, containing three adult skeletons and three human-effigy incense burners, was in front of the structure in line with the central passageway; another was under the central bench. The latter burial held three child skeletons and a collection of pottery whistles representing human figures, animals, and birds. After this building was excavated and mapped, the masonry walls and plastered floors were solidified by Strömsvik.

Other excavations were carried on in various structures in Squares Z and AA where the possibility of finding burials was indicated by depressions or where part of a buried vault was showing. In several instances burials were exposed; in others, nicely constructed stone vaults were found with no skeletal remains. One of the best-built vaults was under the west bench of a large dwelling with stone columns (Structure Z-4-b). Here the vault, which was partially corbeled, was entered through a narrow passageway leading from the terrace at the west end of the building. In this case the vault was filled to the spring of the arch with earth, stone, and pottery, and the passageway was completely filled.

During the three years in which excavations have been carried on in house-type structures, pits have been dug in every location associated with them. Burials were found in benches, passageways, front and back rooms, and terraces. There does not seem to have been any definite rule about burying the dead, for not only did the location vary, but also the method.

Sometimes the remains were placed in well built tombs, sometimes in simple cists put through the floors of structures. There is no doubt that in most cases the well made vaults were constructed at the time of building the house, with the idea of eventual use for burial purposes.

Although little grave furniture was found with burials this season, some interesting finds were made, including several copper rings—three still on one of the phalanges of a skeleton—and a pair of copper tweezers. The recovery of parts of pottery figurines with articulated arms and legs was also interesting.

Sherds of the Mayapan Period were present in all the samples recovered by Ruppert and Smith. In the more elaborate house types excavated by Thompson and Proskouriakoff there was a small percentage of black-on-cream ware which was not represented in pottery from dwellings outside the proximity of the main ceremonial group. It is possible that the people living in the more pretentious dwellings used this type of pottery. Excavation should throw more light on this subject.

During the season four sites, Chun Uc, Chan Pixya, Haol, and Jaba, within easy walking distance of Telchaquillo were visited by Ruppert. They are small ruins located near cenotes and all have house types like those found at Mayapan.

#### MAPPING AND EXCAVATIONS AT MAYAPAN

TATIANA PROSKOURIAKOFF

The map of the Main Group begun last season was completed this year with the assistance of Strömsvik, who performed all the excavations necessary to uncover building plans. The area covered now includes about 130 structures.

The extension of the map has not modified substantially the architectural picture as it was outlined in 1953 (Year Book No. 52, pp. 264-268). The colonnaded hall remains the dominant building type at Mayapan, with twenty-two examples mapped, of which the larger and the more

elaborate tend to occur in the southern half of the Main Group. Residential use of these buildings is suggested in a number of cases by the close association of dwelling-type structures, which seem to be built to provide household service for the halls. This greatly enhances the possibility that the colonnaded hall of Mayapan is a counterpart of the Mexican "Telpuchcalli" (bachelors' house) or of the "Calmecac" (monastery). Almost every hall has a central ceremonial feature, such as a niche or an altar, interrupting the rear bench. Often this central feature was later enclosed in a small shrine; sometimes there is a shrine in the center of the front stairway. In at least nine instances, a platform, shrine, or small temple stands in front, facing the colonnade.

Temple architecture at Mayapan reveals two types of plan and a number of variations. The Castillo, the partially vaulted Structure Q-80, and Structure Q-95 are prominent temples, each of different design. Structures Q-152 and -214 both are round in plan, but differ in size, interior arrangement, and roof construction. A more uniform group is formed by Structures Q-58, -143, -159, and -218, all pyramid-temples with grotesque columns probably ending in serpent tails. These temples answer the description of the "Cu" or "Teupan" of Mexico. Their number at Mayapan recalls the traditional fourfold division of Mexican towns as well as the four "divisions" or lineages mentioned in Maya history. In front of each are a shrine and platforms centered on the axis of the façade. The second type of temple plan is characterized by a lower substructure, two plain columns in the doorway, and two interior piers. Structures Q-82, -55, -88, -153, and -202, representing this type, are less prominently placed than the pyramid-temples and do not have subsidiary structures.

The 36 temples and colonnaded halls, together with Structures Q-142a, -158, and -163a, the nature of which is not clear from the visible remains, can be considered the

major civic and religious buildings of the central group. There are numerous minor buildings that yield less readily to classification. What we call a shrine is typically a one-room building raised on a small platform, facing and centered on a temple or colonnaded hall. Identical small buildings, however, occur independently; on the other hand, subsidiary structures have every degree of elaboration and some are better described as small temples. A general class of subsidiary and minor buildings of one or two rooms would include 23 structures of known plan. Five of these are subsidiary temples or elaborate shrines (Q-141, -148, -149, -155, and -201); six are smaller shrines (Q-69, -71, -89, -90, -98, and -216); 12 are one-room buildings of doubtful function, some on independent platforms and others in some way attached to larger structures. Structures Q-61a, -82a, and -157a are tiny constructions even smaller than the typical shrine, built possibly to house a single idol. The interior of the largest is hardly more than a meter square.

In addition, there are 21 platforms of various kinds, some of which may have supported small superstructures with plans not now apparent. Eight of these are centered on larger buildings, and one, Structure Q-84, may have served as a platform for monuments. Ten other minor structures are too fragmentary to identify, and two sizable platforms, Q-152c and Q-160, have only traces of masonry walls and very little debris to indicate a superstructure. They may have served as foundations for perishable buildings, or perhaps they were not in use at the time of the city's abandonment. Sixteen dwelling-type houses and platforms associated with them complete the total of 114 structures in the ceremonial group. Sixteen others mapped belong to adjacent house groups. The area covered is about 200 by 300 m. This is a very crowded arrangement as compared with plans of earlier ceremonial centers, which are designed more spaci-ously and on a larger scale. It undoubt-



edly results in part from the space limitations set by the city wall and in part from a decentralized ritual organization serving a multiplicity of cults.

Thompson's excavations in Structure Q-208, a dwelling-type building, uncovered some fine ritual objects, and architecture that rivals in scale and quality that of many public buildings. The question arises whether such structures were homes of the aristocracy, or if they were community ritual centers on the order of the Mexican Calpulli. There are several imposing groups of such houses in the immediate vicinity of the ceremonial center that were not covered in the house survey (Year Book No. 52, pp. 256-258) and are indicated on the map of the site with conventional mound symbols. One such group was cleared of bush this season, and minor excavations were made to clarify its plan.

The group is about 200 m east of the Castillo and is one of two that are almost identical in plan. It is built on a natural rise in ground, terraced on all sides to form a raised court. Three houses (R-85, -86, and -87), set on high foundations, enclose the court on the north, west, and south; at the southwest corner steps lead down to a vaulted passage which provides entrance and exit from the court under one of the houses. The building plans are typical of the Mayapan dwelling, with an open front gallery and a range of rooms in back. The galleries, however, are unusually wide (that of Structure R-87 exceeds 4 m), and the heavy columns are widely spaced. What masonry can be seen is superior to any observed in the Main Group.

On the east side of the court, a less solidly built house, Structure R-88, stands directly on court level. In front of it are two small shrines. One of these, Structure R-90, facing west and centered on Structure R-86, is built on an extension of an earlier terrace. The other, Structure R-89, facing in the opposite direction, is built against this terrace at a lower level, and is approached by a short flight of steps leading into a small areaway. Two

"column" altars with carved moldings and a cylindrical stone were lying in the court, apparently moved from their original positions.

It was obvious that someone had previously excavated in this group. A pit had been dug destroying the head of the stairway leading into the vaulted passage. Another was noted in the doorway of the southeast room of Structure R-88, and a third in front of the shrine R-90, where one of the "column" altars probably stood. In clearing this pit, we found a small cache bowl representing a turtle and containing a fine obsidian flake-blade in place just under the surface of the court. A fourth early excavation was a trench through the northeast room of Structure R-85, breaking the medial wall and passing into the bench of the front gallery. It is inconceivable that this was done by an experienced archaeologist or by the departing Maya. The holes were patently made in search of caches and objects of value, but there is little hope of learning when and under what circumstances.

The lower shrine, Structure R-89, had also been entered; there was a pit inside it against the rear wall, where normally an altar would be placed. Afterward, the areaway had been filled with stones, many of which are carved in the Puuc style. Sizable trees had grown up in the areaway since it was filled, but the growth of the Yucatan bush is so rapid that this hint of the age of the excavations eliminates only the past few years.

Unlike most buildings at Mayapan, R-89 still retains its roof intact. The construction is something in the nature of a very crude vault, formed by a corbel of several layers of irregular slabs projecting from the three free-standing walls, and supporting one very large and one smaller capstone. The door is spanned by a stone lintel, and the upper façade is a single three-member molding probably interrupted by a central decorative feature. There are traces of secondary construction on the roof. In front of the shrine are two stone rings set

into the floor of the areaway, which is drained by a small rectangular opening in the floor near the northeast corner. The areaway is secondary to the shrine, which in turn appears to be later than the terrace against which it is built. There are clearly several building periods which involve other structures in the group, but it was not possible this year to investigate the sequence in detail.

Minor investigations included the removal of debris from the main stairway leading to the court at its northeast corner, and from some of the rooms of Structure R-88, which has a rather unusual plan that required clarification. Partial clearing of the stairways to Structures R-86 and -87 and to the vaulted passageway showed at least two sequent building operations, and a buried terrace in the middle of the court suggested a radically different arrangement of the group at an earlier stage. It is hoped that more excavation can be carried on in this group, for it shows promise of yielding a determinable sequence of constructions containing ritual artifacts of high quality.

#### EXCAVATIONS IN MAYAPAN

EDWIN M. SHOOK

The 1954 field season witnessed the continuation of a program to investigate systematically one or more examples of each type of building found within the principal ceremonial group at Mayapan. This study, initiated in 1953, engaged the efforts of most of the field staff and neared completion by the end of the present season. As part of the general plan of investigation, Shook excavated the Castillo (Structure Q-162) and a round temple (Structure Q-214). He also made stratigraphic tests around these units for data bearing on the growth of Mayapan and the evolution of ceramics during the major occupation of the site. The pottery from these excavations was studied in conjunction with that recovered by Irving, Pollock, P. E. Smith, and Winters from other ceremonial buildings.

The most prominent architectural feature at Mayapan is the so-called Castillo or Temple of Kukulcan. It is a terraced pyramid, approximately 15 m in height, with a balustraded stairway on each of its four sides. Tightly clustered around the pyramid are the numerous lesser temples, colonnaded buildings, shrines, houses, and platforms which form the civic and religious center of the ancient city. Several of these units, built after the Castillo pyramid, abut or overlie its lower terraces.

The study of Structure Q-162 established the existence of an earlier, buried pyramid entirely enclosed by a later one. The inner unit, representing one of the first temples erected in the Mayapan Period, apparently differed only in size from the final structure. Both had plain, battered terrace walls constructed of the typical Mayapan block-and-slab masonry. Though we probed to a depth of 2 m through the dry rock fill below the top floor of the later temple, we did not encounter an earlier superstructure. It appeared likely that the building had been razed by the builders of the second temple, as was the case in Structure Q-58 (Year Book No. 52, p. 269).

Our excavations at the north and west base of the Castillo disclosed that only two successive court floors had joined the earlier pyramid, whereas at least nine floors, each representing renewed building activity, turned up to the stairway of the outer unit. Also, no less than seventeen successive lime floors were found in the late temple on the summit of the pyramid. These numerous remodelings and the pottery sealed below the primary floor indicate that the construction of the later Castillo took place quite early in the Mayapan Period.

The temple, similar in plan to the Castillo in Chichen Itza, has its principal entrance on the north side. Here a triple entrance formed by serpent columns leads to a transverse chamber. Access to the central sanctuary is gained through a single doorway from this northern chamber. Surrounding the east, south, and west



sides of the inner sanctuary, but with no interior means of communication with that room, is a narrow continuous gallery with a single exterior doorway centered on each of the pyramid stairways. The masonry walls of the temple supported a flat beam-and-mortar roof. The roof type was evident from the composition and amount of fallen debris in the rooms, the absence of stones adequate for vaulting, and particularly from the width of the inner sanctuary. This chamber originally had had a masonry altar against the center of the south wall, opposite the doorway. Before the collapse of the temple roof, which buried the floor and lower walls to a depth of 1.25 m, the masonry altar had been torn out and a hole dug 50 cm below it. We assume this destruction was done, at the time Mayapan was abandoned, by looters in search of offerings known to be contained in the altar. Why they took the trouble of refilling the hole with the debris from their digging is not clear. Among this disturbed material we recovered shell beads and ornaments, obsidian flake-blades, chipped flint implements, and many pottery fragments from small vessels and censers. The pottery objects may have been accidentally broken, and the small artifacts of shell and stone overlooked or not valued by the ancient looters.

Most of the seventeen floors representing different refurbishings of the temple bore evidence of much burning. The heaviest concentration of burning took place on the north-south axis of the building from the front terrace to the altar in the sanctuary. Despite the blackened areas and lenses of copal incense between certain floors, there were relatively few censer fragments on the temple floor or at the foot of the north stairway. Several other temples (Structures Q-58 and -82, for example), colonnaded buildings (Structures Q-81, -97, -151), and shrines (Structures Q-69, -71, -90) were found to be littered with broken censers. Perhaps the Castillo, being the most important temple of the city, was carefully

tended and kept clean, whereas slovenliness was permitted in less sacred buildings.

No burial shaft was found in the Castillo. A tapered stone block of a type associated with sacrifice was, however, lying at the base of the north stairway and suggests that sacrifice may have been made before the temple.

The next building investigated was a round temple, Structure Q-214, located about 100 m south of the Castillo. It occupies the central position on the west side of a small plaza and faces east toward a temple with serpent columns (Structure Q-218) on the opposite side of the plaza. Structure Q-214 consists of a circular outer wall with the interior space divided in half by a partition wall. A single doorway provides access to the front room; another in the partition wall, to the rear chamber. These rooms, small and semicircular in plan, apparently had been vaulted. A burned area 50 cm in diameter on the floor at the center of the rear chamber and opposite the doorway indicated some ceremonial use of the temple. Also, many fragments of human-effigy censers lay on the floor near the back wall, and quantities of such pieces came from around the base of the platform supporting the temple.

The 2-m-high platform had square corners on the east or plaza side; on the back or west side each corner was rounded. A stairway with plain flanking balustrades led from the plaza level to the broad terrace in front of the temple. Originally, the flight of steps was uninterrupted, but at some time after the primary construction, the lower central part of the stairway had been cut into and a miniature shrine room erected there. This tiny roofed chamber housed a stone idol depicting a bare-breasted seated female. The statue sat against the back wall of the shrine facing the doorway. Below the sculpture we discovered an offering that consisted of several jade and shell beads in a small effigy vessel. The female figure, the first of its type to be found in Mayapan, may be the important Maya goddess Ix Chel, and thus may

throw a new light on the function of the round temple.

Stratigraphic excavations were made in a deep midden of temple and household rubbish off the southwest corner of Structure Q-214. These produced a sample of sequent materials for the latter half of the site's occupation.

Of more value in establishing ceramic chronology from the beginning of the Mayapan Period were the excavations between Structure Q-77 and the north stairway of the Castillo. Here a vertical sequence of thirteen plaza floors from bedrock to the present-day surface spanned the life of the site during the Mayapan Period. Of the oldest material, in the soil overlying bedrock and sealed by the earliest constructions, 94 per cent was of Mayapan Period types, the remainder being weathered fragments of Puuc and other pre-Mayapan types. In other words, the small amounts of earlier material in the old surface soil indicate habitation in the immediate vicinity prior to the Mayapan Period occupation, but there was no evidence of any construction's having existed in the area tested until the establishment of the political capital of Mayapan.

The major pottery types of the Mayapan Period were current in the oldest level and, except for black-on-cream, continued with minor changes throughout the sequence of floors, or for that matter until the abandonment of the site. Pottery censers are present from the beginning, but the human-effigy hourglass type does not appear before the latest constructional activities and is most common on the surface floors, a condition true of every type of structure so far investigated in Mayapan.

#### EXCAVATIONS IN CEREMONIAL STRUCTURES AT MAYAPAN

PHILIP E. SMITH

Virtually the entire season was devoted to the excavation of three ceremonial structures in a relatively concentrated area of

the Main Group. The most important of the three was a temple, Structure Q-153, adjoining Cenote Ch'en Mul, which is at the eastern base of the Castillo. Following the excavation of this temple and its adjacent constructions around the mouth of the cenote, two smaller, single-room buildings (shrines or subsidiary temples) about 30 m east of the cenote were investigated. The work in general was a continuation of the effort begun last year to determine by intensive digging the nature of certain ceremonial structures and their functions in the life of the Main Group.

Owing to the obviously significant ritual position of Cenote Ch'en Mul in the main ceremonial center of Mayapan, it appeared desirable to try to determine the nature of the adjoining temple and whether it would give any indication that a cenote cult had been fashionable at Mayapan as at Chichen Itza. A Spanish missionary who visited Mayapan less than 50 years after the conquest of Yucatan reported that according to local tradition victims were thrown into this sink after being sacrificed to the gods. Digging inside the cenote during the previous season (R. E. Smith, 1953, C.I.W., Current Report 5) established that water holes still exist and that they had been used in the Classic as well as in the Mayapan Period; but nothing pointing to the ceremonial casting-in of humans or objects, as had occurred at the Sacred Cenote at Chichen Itza, was found by the investigations inside Ch'en Mul.

The mouth of the cenote is about 10 m in diameter and roughly circular. The temple is built on the south side, less than a meter from the present rim—so close, in fact, that a good deal of the collapsing superstructure has fallen into the cenote. The structure is approximately 8 m square.

Around the low substructure runs a cornice about 25 cm high; the wall below this cornice does not drop vertically, but has a definite batter or sloping zone. The plan of the building is not unusual and is somewhat reminiscent of the Xtoloc Temple at Chichen Itza and the Cenote House



at Tulum. The entrance faces east, where a balustraded stairway leads to a low, gently rising terrace, at the rear of which stand the plinth of the building and the remains of two columns flanked by door jambs. The column drums are of the low, rough type common in Mayapan; four stones of the south column and five of the north are still standing, and part of the originally thick plaster coating can still be seen on the bottom stones. Thus the original effect must have been that of a triple-entrance doorway to the temple interior.

One suspects from the absence of a thick layer of fallen plaster on the surface of the interior that this building did not have the common beam-and-mortar roof, but perhaps was thatched. Flakes of red-painted plaster indicate the probable color of the walls.

The altar, against the back wall of the building, behind two interior piers, was found to be undisturbed; the cache, in the center and very near the surface, consisted of two badly crushed Red ware tripod bowls placed lip to lip and containing a partly burned ball of copal.

The temple seems to have had only one primary building period, although renovations to the superstructure in the way of varying floor levels and bench construction occurred from time to time. Excavation under the stairway showed that the terrace wall is built directly on the thin layer of sterile earth overlying bedrock, and the same is indicated on the west side. The terrace between the stairway and the front of the building was thoroughly tested by means of a pit extending to bedrock, but under the two top plastered floors only heavy rock fill was encountered.

Last season, burial cists were found in most of the shrines and temples explored in the northern part of the Main Group. It was thought likely that this temple might hold a similar cist or shaft, but nothing of the kind was found. Thus the method of disposition of the bodies of sacrificial victims, if there were such, is

still uncertain. As was mentioned before, no skeletal remains were found in the cenote, although this does not rule out the possibility that corpses were thrown in and later removed. In fact, the closest indication of human sacrifice is a femur found on the west edge of the cenote, a highly doubtful piece of evidence.

The pottery found on the surface and in the various trenches is of the common Mayapan types. No vessels were found intact. Although a number of utilitarian ware sherds were uncovered, the largest percentage was, as might be expected in a structure of this nature, of ceremonial ware, mainly effigy-type incense burners. The exterior northwest corner of the temple, at the cenote edge, seems to have been used as a dumping place, for a great number of animal and bird bones and of sherds (mainly of incense burners, and fairly large) were dug out. From this dump too came a small turtle of a jadelike green stone, well polished, with a somewhat stylized head and body.

On the west and north sides of the cenote, retaining walls of rough stone had been constructed; the east side, from the temple to a ramp adjoining the long colonnaded hall, Structure Q-151, was bounded by a low platform, the present height of which is less than a meter. Although from the surface this platform appears to be a single structure, it really represents construction of two different periods. The northern half is contemporary with a low stairway which was later covered by the ramp mentioned above; originally this part of the platform stood free, but later it was joined to the northeast corner of the temple by a southward extension of the platform. When this was done, it was necessary to make a drain under the south section to provide for drainage of the plaza floor. No attempt was made this season to clean the drain entirely of the rubbish which choked it, but many sherds, mostly of incense-burner type, and a number of large lumps of burnt copal were recovered from the aperture

on the cenote side. There can be little doubt that the platform had some part in the ceremonial functions centered about this important cenote.

The next unit investigated was a shrine or subsidiary temple, Structure Q-149, about 30 m southeast of Cenote Ch'en Mul and facing east toward a temple, Structure Q-143. It was decided to include in these investigations a small, low platform about 5 m east of, and centered on, the stairway of Q-149.

The shrine is typical of Mayapan architecture in most respects, consisting of a single room built on a substructure of moderate height. There is the usual plinth, but no columns, entry being made through a doorway formed by two monolithic jambs which still stand. To judge by the thick layer of fallen plaster inside the shrine, the roof had been a beam-and-mortar affair. Several coatings of plaster were visible on the lower parts of the door jambs, the last showing small patches of painted designs in red, black, and green.

Inside the shrine the floor was cleared, and the altar, which had been centered against the back wall, was found to have been ripped out; its original position could be traced by the upturning of the floor around it. A small hole had been dug below the base of the altar through an earlier floor, and the destruction may well have been the work of looters.

When the terrace in front of the plinth of the building was being cleared, a large sculptured stone head was found. From near by came a hand and a leg of the same proportions. The head resembles two others recovered last season near the base of this structure, and it is probable that all three were at one time part of sculptured figures placed, perhaps, on the roof or over the doorway.

In order to ascertain whether this shrine, like others in Mayapan, contained a burial cist, a pit was excavated in the terrace before the plinth. No indication of such a construction was found.

Digging around the base of the balus-

traded stairway revealed that above the original plastered levels of the plaza around Structure Q-149 there had been a rough stone layer which served as a base for a later floor. Built directly over this layer is a secondary wall which extends around the rear half of the shrine's substructure. Roughly U-shaped, about 60 cm thick and with a present maximum height of 1.65 m, it extends around the west side of the substructure and about halfway around the north and south sides. It is hard to believe that its purpose was to beautify the shrine; it may have been erected as a reinforcement for a weakened wall of the substructure.

The small platform in front of the stairway of Structure Q-149 is approximately square, about 1.5 m on each side, and at present only one course of stones high. It rests on the stone layer covering the original plastered floors of the plaza, and thus some idea is obtained of the building sequence. An excavation was made into the small earth-filled depression in the center, but nothing was uncovered. The purpose of this platform is uncertain; it may have been an altar-like construction, the base for a figure, or the foundation for a sacrificial stone such as is known to have been placed sometimes before stairways.

The other shrine or small temple excavated, Structure Q-148, is also a single-room building on a substructure of moderate height. In spite of its proximity, it does not seem to have had any ritual connection with either of the structures just described. Rather, it appears to be aligned with the colonnaded hall, Structure Q-151, for its stairway, which faces north, is directly opposite the main altar of the hall.

Unlike Structure Q-149, and for that matter the vast majority of shrines at Mayapan, this building had a triple-entrance doorway. A number of Puuc-style mask stones, some badly shattered, were observed on the surface even before clearing, and when the area between the door jambs was excavated, the lower sections of two pillars faced with similar elements



came to light. The original height of each pillar, as estimated from the fallen stones recovered, must have been at least 1.5 m. The stones, which are roughly the sector of a circle in plan, the curved surface being the face, are laid one above another to form the front of each pillar. Some of the stones have designs and others are plain, but actually they were covered with thick coatings of plaster to give the impression from the outside of solid columns supporting the door lintel.

Centered against the back wall of the room was a masonry altar. A small flake of gold the size of a fingernail, bearing a faint design, was found in the debris near by. Also near the altar were discovered several large pieces of charred beams. These not only indicate that the shrine had a beam-and-mortar roof, but lend some support to the old tradition of the destruction of the city before its sudden abandonment. The surface of the altar had been destroyed, and the presence of more fragments of charred wood in the earth inside makes one suspect that the altar was open when the roof burned. If so, and if looting had been the intent, the operation was not entirely successful, for between two front stones of the altar was found a small effigy vase containing two jade and three shell beads, a tiny fleck of copper, and a small jadelike flake of a mosaic. A copper bell and another shell bead recovered near by may also have formed part of the cache.

Excavations both inside and outside the shrine revealed several floor levels, which give some idea of the various changes in the original structure. A trench was dug in front of the building into the rock fill of the substructure, but just as with the temple by the cenote and the other shrine, no burial cist or shaft was found.

Digging around the walls of the substructure uncovered an unexpected secondary wall or facing running all along the east, south, and west sides. Like the secondary wall around the rear part of Structure Q-149, this construction may have been a means of changing the original

design of the shrine, or it may have been a reinforcement to support collapsing inner walls. A similar structure (Q-154) a short distance southwest seems also to have a secondary wall around it. The thought occurs that this architectural feature may have been fashionable or significant at some period in this section of the Main Group.

#### EXCAVATIONS IN MAYAPAN

WILLIAM N. IRVING

The greater part of the field season was spent in the excavation of Structure Q-151, one of the larger colonnaded halls in the main ceremonial group at Mayapan. During the later part of the season a large residential unit, Structure Q-231, was surveyed, and a plan was made showing such details of construction as could be discovered by making limited spot excavations. Finally, observations were made and recorded on the techniques used at Mayapan for working in flint and obsidian.

The colonnaded hall, Q-151, by virtue of its location near the Castillo and Structure Q-152, the largest round temple at the site, and Cenote Ch'en Mul with its near-by small temple, is a prominent member of the main ceremonial group. Its excavation was expected to shed some light on the plan and the nature of the activities associated with such halls in general, and on the use of this building in connection with public functions in the center of the city. The structure was also considered suitable for partial restoration as representative of Mayapan colonnaded halls. Finally, there was promise that stratigraphic cuts would produce information to permit a finer demarcation of phases within the Mayapan Period.

The entire front of the building and substructure were cleared of fallen debris to the most recent levels of construction, from the west end to a point some 3 m east of an enclosed medial shrine set in the bench that runs along the rear wall. This excavation exposed a gallery that had formerly

had a beam-and-mortar roof supported by two rows of columns and short wall sections, the bench and medial shrine just mentioned, and a shrine or altar at the center of the stairway. A notable feature is the installation of Puuc-type façade masks on masonry piers set between Columns 3 and 4 and between Columns 7 and 8 of the ten columns in the front row. The city abounds in sculpture and stone decorative elements referable to the Puuc style, but this is the first instance of their having been re-used as originally intended but in a Mayapan Period building.

Of particular interest are the results produced by the stratigraphic trenches. One, in a section of the filled passage formed by the rear wall of the colonnaded hall and the substructure of the round building, Q-152, was excavated to bedrock (ca. 2.5 m) by 50-cm levels. The pottery analysis, undertaken by Shook, showed that the hall was built rather earlier in the Mayapan Period than had previously been supposed, well before the sudden proliferation of effigy incense burners and the development of certain late forms of utilitarian ware. The passage had apparently been filled alternately by the dumping of rocks and lime mortar, and by debris, presumably from the platform of the round building, which included human corpses and utilitarian as well as ceremonial pottery. The relationships of natural soil and substructures seen at the bottom of the trench indicate that construction of the colonnaded hall postdated that of the round temple.

A second stratigraphic excavation was made in the corner formed by the juncture of the west wall of the substructure of the round building and the rear wall of the colonnaded hall. This revealed a short bench along the former substructure which had been built over a deep (ca. 1 m) accumulation of midden material. The significance of this midden, in an exposed spot next to two prominent public buildings, is not yet fully understood.

A trench cut through the center of the

colonnaded hall from the bedrock in front of the medial stairway to the shrine at the rear showed a building sequence marked by at least nine floors; several significant changes in the central altar at the rear of the building and in the shrine, which produced fragments of stucco and stone sculpture and a wealth of broken incense burners; and apparently one transposition of the supporting columns. It is hoped that a detailed comparison of these features with their counterparts in other similar halls will shed light on the development of this type of structure and perhaps on its intended use.

Structure Q-231, located less than 200 m from the Castillo, is considered to be a residential group, one that might well be described as a palace. It consists of two large dwelling-type structures on high platform mounds, and two smaller buildings on low platforms, the whole arranged in the form of a quadrangle. The small amount of digging done in connection with mapping its gross features suggests that the group would be an interesting one in which to continue excavations.

#### EXCAVATIONS IN MAYAPAN

H. E. D. POLLOCK

During the past field season Pollock undertook a number of limited excavations at Mayapan to ascertain needed architectural details and the chronological relations of certain buildings. The first such excavation was concerned with the Castillo and Structure Q-163, a colonnaded hall that lies at the foot of the west stairway of the larger structure. A distance of less than a meter separates the east end of the hall from the lowest step of the stairway, providing an excellent opportunity to discover the relative time of construction of the two buildings. Our excavations revealed a series of eight floors, the lowest of which appeared to be contemporaneous with the construction of the stairway of the Castillo. This floor and the next above it ran beneath the plinth of the colonnaded hall.



Floor 3 and all higher floors are well above the level of the bottom of the plinth. Unfortunately, they are broken, within the area of our excavation, just short of the plinth course of the hall, a condition which can best be explained by root action, but which may hide the fact that the builders of Structure Q-163 cut through one or more of these floors at the time of construction of the building. What is clear is that the colonnaded hall, Structure Q-163, is contemporaneous with, or later than, Floor 3, and was built later than the west stairway of the Castillo.

The next two excavations involved the determination of roof heights of colonnaded halls. Owing to the relatively unstable construction of this type of building, little remains to give an indication of the proportions and general appearance of such structures in their original condition. At the east side of the Castillo a colonnaded hall, Structure Q-161, is built against the terraces of the pyramid, and on the west side of the pyramid another structure, probably a colonnaded hall, is similarly located. Because of the support provided by the terrace walls of the pyramid, these locations were likely spots to look for fragments of roofs still in position.

Our work at the west side of the Castillo was successful in uncovering what clearly was a section of the roof of the building below. This fragment was preserved in the angle between the south wall of the west stairway of the pyramid and a terrace wall. Excavation of the plinth of the building thus provided a height from floor to roof cap. Unfortunately, the great depth of debris against this side of the Castillo obscures the precise character of the building that had stood here, but there is some reason to believe it was a colonnaded hall.

Our work at the east side of the Castillo pyramid was less conclusive. No surely identifiable roof cap could be found. There were indications, however, that the roof of Structure Q-161 was on the same level as, and merely a continuation of, the floor of the upper surface of the second

terrace of the pyramid. This gave a height above the floor of the colonnaded hall of roughly 3.7 m, a figure closely approximating that determined at the west.

Structure Q-159 is a small pyramid-temple immediately south of the Castillo. Although very little of the superstructure was visible before excavation, there was a columnar entrance at the east and the bare suggestion of a battered lower zone on the walls of the temple. The latter feature seems to be one of the characteristics of serpent-column temples at Mayapan, and the existence of a fallen fragment of a stone serpent tail on the northwest slope of the pyramid added to the possibility of the building's being of that type. Excavation quickly proved the existence of the battered lower wall zone and that the two columns in the entrance to the temple carried stucco figures at their bases. Instead of the expected conventional serpent heads, however, these figures were four-footed monsters, possibly Earth Monsters, although the stucco was not sufficiently well preserved to make identification sure. The finding of part of a stone serpent tail beside one of the doorway columns, a fragment that fitted the one previously noted, and of a second serpent tail off the southeast corner of the pyramid left little doubt that the doorway supports had been completed at the top in conventional serpent-column fashion. In other words, we had here a new and previously unknown form of decorative doorway column, part serpent, part monster. This is a type so far known only at Mayapan.

Further excavation developed a single-room temple building with an altar centered against the rear wall. In front of the altar was the stone figure of a turtle, fallen from its original position. There was nothing distinctive about the terraced pyramid which carried a balustraded stairway on the east, in which direction the structure faced. Pottery from within the building was mainly of effigy incense burners; and a single sherd of Red ware from beneath the floor, where only three sherds were

recovered, would seem to prove the temple to be of the Mayapan Period.

Another small excavation was directed to establishing the sequence of construction of three adjoining structures some 50 m east of the Castillo. Structure Q-143, a small pyramid-temple with serpent-column doorway, is clearly older than Structure Q-142a, which abuts it at the north. East of Q-142a is the colonnaded hall, Structure Q-142, the west wall of the latter built against the rear wall of Structure Q-142a. From surface observation it was impossible to tell which of these two buildings was the earlier, but it appeared that the determination of this fact might yield a sequence of three buildings, because of the relationship of the pyramid-temple to Q-142a, all of different types.

Excavation readily revealed that the west wall of Structure Q-142 was erected later than Q-142a. A closer examination of Q-142, however, showed that the west end of the building almost surely was a later addition to the main body of the colonnaded hall to the east. We were thus unable, without considerably more extensive excavation, to tie the primary part of Structure Q-142 into our sequence. Floor levels suggest that the original building is contemporaneous with, or later than, Structure Q-142a, but this was not proved. A tentative sequence of construction might be, from early to late, Structures Q-143, -142a, -142, west addition to -142.

Lastly, work was undertaken at the northern terminus of the city's principal sacbe, or artificial road, which connects two groups of structures roughly 400 m apart. Both groups are of the courtyard variety, the northern in Square R, the southern in Square Z, and probably were domiciliary in character, although they may possibly have served as community ritual centers. Our excavations were designed to discover the relative time of construction of the sacbe and the architectural assemblage at its northern end, and if possible to ascertain the period of

the road in relation to the time of occupation of the site.

Although these excavations started as a strictly limited operation, the existence of a complex system of superimposed floors, platforms, and terraces led to turning over considerably more dirt than originally was contemplated. Approximately two weeks were spent at this location, and at the end there were several unsolved but pertinent questions that only much more widespread digging could answer. In the main, however, our purpose was achieved. It was perfectly clear that the building of the sacbe was at or near the end of the sequence of constructions uncovered by our excavations, and there were good indications that the road was late in relation to the entire group. Pottery from beneath the sacbe not only was almost purely of Mayapan Period, but contained an appreciable percentage of wares known to occur late in that period. It thus develops that the northern end of the sacbe, and presumably the entire road, was built late in the history of the city, a fact that was not without surprise, as at the time of these excavations no examples of Maya roads from this late period were known. It later developed that the explorations of Sanders this season on Cozumel Island off the east coast of Yucatan disclosed two roadways that may be of Mayapan Period.

#### EXCAVATION OF RESIDENTIAL-TYPE STRUCTURES IN MAYAPAN

J. ERIC S. THOMPSON

Structure Q-208, situated about 80 m south of what may be the southern edge of the ceremonial center, was chosen for excavation because, from what was visible above ground, it appeared to be the residence of some important person, a type of structure not previously explored. The remaining structures were examined to see if, because of their contiguity, any functional relationship to Structure Q-208 could be established.

Structure Q-208 consists of one long



room with an open front (north) with four columns. Doorways in the back wall lead to three small and narrow rooms, of which the central one served as a shrine. On the east end of the structure there is a fifth room, without direct communication with the other rooms. Walls are very largely built of re-used dressed stone of the Puuc Period, of excellent quality, and one of the four benches of the front room is decorated with squat spool ornaments typical of Puuc architecture. Heavy layers of fallen mortar showed conclusively that the roof had been of the beam-and-mortar type.

Great quantities of charcoal, some pieces of which retained the shape of sections of poles, were scattered through the fallen mortar and in some cases underlay it, supplying conclusive evidence that the building was burned. Because a beam-and-mortar roof could hardly burn and collapse as the result of an accidental fire, we can feel fairly confident that the destruction was deliberate. As ceramic evidence shows this to have happened at or near the end of the occupation of Mayapan, it is reasonable to suppose that the burning took place at the fall of Mayapan (circa A.D. 1460). The absence of any noticeable accumulation of dirt between the fallen mortar and the floor, and the presence of offertory vessels on the floor at the entrance to the shrine, indicate that the building was in use at the time of its destruction.

There was no evidence that food was prepared or cooked in the building, but in view of present-day Maya usage (there is often a small hut for cooking behind the main house) that was hardly to be expected. On or slightly above the floor were several artifacts, including two finely polished celts (one of fine-grained greenstone, the other perhaps of basalt), a flint point, and two finely chipped arrowheads, one of obsidian and the other of flint. Unless the archaeologist in charge is directly looking over each shovelful of earth excavated, it is difficult to know the exact depth of any stray find. Luckily, in the

case of one of the celts, it is established that about 4 cm of roof mortar separated it from the floor. As it was close to the base of one of the columns, it is a reasonable conclusion that it was dislodged from some shelf or niche in the wall above the columns and lintels; had it been in full view, the burners of the building would surely have taken it. The second celt also was close to a column, but it is not certain whether it was directly on the floor or in the fallen mortar. There was no domestic pottery on the floor; many, if not all, of the sherds in the debris had formed part of the ceiling mortar.

At the entrance to the shrine two effigy vessels representing the "diving god" stood before a fallen sculpture of what may be a variant rendering of the same deity. This is, for Mayapan, of remarkably fine workmanship. The cult of the diving god may be of Mexican origin. Beneath the building was an earlier structure of about the same size, and beneath the floor of that were the remains of several small children, one of whom had anklets of copper bells. Above the burials were many sherds of incense burners. Among these were fragments of five bearing effigies of the Mexican god Xipe Totec, easily recognized by the flayed skin he wears. Bishop Landa describes the flaying sacrifice, but as this ritual also had a part in ceremonies of certain Mexican goddesses, it had not previously been known beyond doubt that there was a Xipe cult in Yucatan.

There was no direct evidence of a functional relationship between the other buildings in this group and Structure Q-208, but in the case of two of them a connection may be surmised.

Structure Q-209, directly east of Q-208, was a small residential structure with two rooms, one behind the other, separated by a medial wall. Each room had two benches divided by a passage which pierced the low medial wall. In contrast to Structure Q-208, Q-209 was constructed of inferior masonry; the walls had been low and the roof of perishable materials. As Structure

Q-209 stood on a platform separated by a shallow passage, only 59 cm wide, from Q-208, and was of later date, it is reasonable to conjecture that it was the residence of inferior members of the household of the chief who occupied Q-209; for it is difficult to imagine that he would permit strangers to build and occupy an addition to his dignified residence. Structure Q-208a was a small low platform standing on a subplatform which underlaid that of Q-208. The platform is separated from the north-west front of Q-208 by only 1.30 m, and faces east. Although its subplatform is earlier than Q-208, the present, simple platform may be of later date. It supported no structure, and no evidence of its use was obtained. Nevertheless, its contiguity to Q-208 and the fact that it faced on the court in front of the latter suggest a connection.

Structure Q-207 occupies a commanding position on a natural hillock immediately south of the east end of Q-208, and faces east. Access was by means of a stairway conforming to the natural slope. The front room had three benches and was open on the east side. Two doorways gave entrance to a long, narrow room behind. This, in turn, had a doorway leading to a shrine room in the rear. Walls were low, and masonry was of inferior quality. A preliminary study of sherds from beneath the floors seems to show that Structure Q-207 was anterior to Q-208. This, together with the eastern orientation of the building and its staircase, argues against a close association with Q-208.

#### EXPLORATION AND RECONSTRUCTION IN MAYAPAN AND VICINITY

GUSTAV STRÖMSVIK

Toward the end of October, when the extraordinarily heavy rainy season of 1953 approached an end, Strömsvik began the previously planned exploration of the vicinity of Mayapan. The purpose of this work was to gain some idea of the extent of Mayapan culture during the principal period of occupation of that city and to

note any ruin of different cultural affinity or of different period. Several groups of ruins were found, the most important being Xcanyulmil, about 4 km south and slightly west of the Castillo in Mayapan; Xpopil, roughly 3 km west-southwest; and Santa Cruz, a little over 2 km south and slightly east. Such groups are usually built near cenotes, each site consisting of a central pyramid structure around which are terraces and smaller mounds. With the exception of Santa Cruz, these sites appear to be of Mayapan Period. Santa Cruz seemed somewhat different. It is more widely spread, and the pyramidal structure is small and seemingly of little importance as compared with those at the other sites. Furthermore, at a platform some distance from the center of the ruins were found potsherds that appeared to be of Puuc type. The site was suggested as offering possibilities in the search for ceramic stratigraphy (see pp. 282-284). Sketch maps were made of the three sites.

With the beginning of large-scale operations at Mayapan the middle of January, Strömsvik undertook a series of small excavations in connection with Proskouriakoff's detailed mapping of the main ceremonial group at the site (see pp. 268-271). This work was designed to develop plans of buildings and hidden details of architecture preparatory to Proskouriakoff's mapping. With the exception of a week given to exploration in Quintana Roo with Pollock (see pp. 289-292), the work continued until the middle of March.

In accordance with an informal agreement made the previous year with the Instituto Nacional de Antropología e Historia of the Mexican Government, Strömsvik took charge of the consolidation and repair of two structures typical of Mayapan architecture. The buildings selected for such treatment this season were a large colonnaded hall, Structure Q-151, just east of the Castillo, and a house-type building, Structure Q-62, adjacent to another colonnaded hall at the northern edge of the Main Group.



Structure Q-151 was excavated by Irving (see pp. 276-277). A little over half of the building was cleared of debris, as it was thought that this part of the structure, which included the centrally placed shrine and altar, would provide a clear understanding of the colonnaded hall. Walls were rebuilt with lime-and-cement mortar to the height that they stood when excavated. Columns were reset to a height less than that of the back wall but sufficiently high to indicate their function. For the sake of permanence, lime-and-cement mortar was placed between column drums instead of the mud mortar that originally had been used. The original 3-5-cm-thick lime plaster covering that had encased the columns was not reproduced. The decorative masks on the façade of the building were rebuilt as completely as available parts allowed.

Structure Q-62, considered to be a good example of simple living quarters in Mayapan, was excavated by A. L. Smith and Ruppert (see pp. 267-268). Walls and benches were reset in the original manner, and the over-all plan—room arrangement, porches, passageways, and benches—was made clear to the observer. The low walls of masonry had once, of course, supported wood-and-thatch construction, which we did not attempt to reproduce, as such material would fall prey to the elements in a few brief years.

This task of reconstruction and the not inconsiderable labor of clearing away the tremendous quantities of debris that had accumulated from the excavations lasted until well into June. The work was interrupted the last week of May to make a second trip, this time accompanied by Berlin, to the ruins in Quintana Roo that had been visited earlier in the season by Pollock and Strömsvik.

#### POTTERY OF MAYAPAN AND VICINITY

ROBERT E. SMITH

The number of potsherds collected at Mayapan during the 1954 field season

totaled nearly 130,000. A very large percentage of these belonged to the late Mayapan Period and were found on or near the surface. Earlier Mayapan Period types (black-on-cream, certain Red ware shapes, and certain styles of incense burners) were located below floors or in deep stratigraphic cuts, most of which were associated with ceremonial structures. The ceramics from ceremonial structures have been discussed by Shook (Year Book No. 52, pp. 271-272). The trends noted by him are essentially the same as those seen in the pottery from elsewhere in Mayapan. Differences worth mentioning are that effigy censers occur in considerably greater proportion in the house mounds, often elaborate, of the ceremonial center than in the simpler house mounds spread over the site, and that black-on-cream pottery forms a small percentage of the types associated with the house mounds of the ceremonial center and is rarely found in those outside. At present, our knowledge of house-mound pottery is limited to the excavation of a few domiciliary structures both inside and outside the ceremonial center. Nevertheless, there is a suggestion that the more elaborate style of domiciliary structure harbors a more elaborate style of pottery, e.g., effigy censers, effigy vessels, and rather large painted figurines. Also it is probable that most of the house mounds excavated belong to the Mayapan Period, since the ceramic types of that time are predominant. In fact, the ceramic types of earlier periods taken from all house mounds excavated from 1952 through 1954 will not form 1 per cent of the total house-mound pottery. On the other hand, the house platforms of the small outlying site of Santa Cruz abound in early potsherds.

The ruins of Santa Cruz are situated about 1.5 km by trail southeast of Cenote Ch'en Carro, which is located in the south-central part (Square Y) of Mayapan. These ruins, which occupy a relatively small area, roughly 200 by 300 m, part of which is used for pasturage and part for growing corn, squash, and peppers, were

discovered and mapped by Strömsvik in November 1953. There are in all eight platforms and a small pyramidal structure. This last and five of the platforms are clustered around a sizable jug-shaped cenote which contains what the natives claim to be the coolest, freshest, and most palatable water in the vicinity. The platforms have been numbered from A to H. Only three were investigated: A, the farthest north; B, just northwest of the cenote; and C, due east of the cenote.

The investigation of Platform A consisted of digging three trenches. The West Trench disclosed the original west wall of the platform to a depth of about 1.5 m. This wall was constructed of large and medium-sized stones and was probably built during the Puuc Period; both the appearance of the wall and the ceramic evidence from inside the platform support this suggestion. The sherds from the three strata in this trench were all of pre-Mayapan Period pottery save for one possible effigy-censer fragment found in the top stratum. The proportion of Formative to Puuc Period types increased from top to bottom.

Perhaps the most instructive of the three trenches was that dug alongside, but not directly next, the north wall. After sinking an exploratory pit well to the west, and finding only Puuc and Formative pottery types except for a single effigy-censer sherd, we prolonged the pit into a broad trench. Two infant burials, both in Puuc-type jars, and two Puuc Medium Slate bowls, apparently associated with an adult burial, were encountered in stratum 2. Very few artifacts were found in the North Trench, all in stratum 2. The ceramic pattern resulting from the three levels excavated resembles closely that of the West Trench with a greater abundance of early types. An interesting fact about this trench is that it appears to have cut through a refuse deposit, at least in the western section.

The East Trench passed through several terraces and finally bisected a single-room shrine at the north edge of the platform.

A number of floors at different levels were encountered. Close to the surface at the south end and above the top floor in the shrine at the north end, two rather crudely made, small Mayapan Red vessels were found, whereas about 1.3 m below the top floor and sealed beneath the earliest and best-preserved floor a formal Puuc Period crypt burial was encountered. This burial contained an adult male skeleton, three Puuc-type pottery vessels, a number of jade, shell, and bone ornaments, and five jaguar teeth. Few artifacts were found in the middle and top levels. The ceramic pattern derived from the three levels in the East Trench differs considerably from that of the West and North Trenches. More Mayapan types occur in the top level and fewer Formative types in the bottom.

Platform B supported several house platforms, two of which, and an altar, were investigated. The latter were enclosed within a walled area in the southwest part of the platform. For the most part the walls appeared to be of postconquest construction, possibly quite recent, but with some sections presumably dating from ancient times. In order to determine the manner in which Platform B was built, as well as to collect a pottery sample, a broad trench was cut into the north side. This North Trench uncovered at least three terraces and two floors. Very few artifacts were found, all from the surface level. The ceramic pattern disclosed by the five levels excavated resembles that of the West Trench in Platform A.

House Platform 1, rectangular in plan and located in the southeast corner of Platform B, was bisected by a trench reaching to bedrock. Just south of the house platform a cache was encountered 68 cm below a fallen monument or, if not a monument, possibly a door or gate jamb. The cache contained two vessels, one a lustrous streaky brown jar, the other used as its cover, a Puuc Medium Slate tripod dish with red floor design. Near the cache but above it, two flint points were discovered, one especially well made. Within



the house platform there was a fairly good floor 32 cm below the surface, and 80 cm below this floor, just inside the south edge of the house platform, a burial with three pots was found. Most of the skeleton, an adult, was lodged under an enormous rock. The vessels, one of which covered the skull, were all Puuc Medium Slate types. The pottery found in the trench totaled only 185 sherds; because of a cave-in during the removal of the burial vessels, some late sherds were mixed with material from the lowest level. The middle level, however, had no pottery later than the Puuc Period.

House Platform 2, rectangular in plan and located just northwest of House Platform 1, was examined by means of a 40-cm-deep trench skirting its northern half. Later a pit was sunk to bedrock off the west side, in the upper 40 cm of which a quantity of Mayapan Period porous gray jar sherds were uncovered. They rested in ash and were surrounded by medium-sized stones, as if some ceremony had been performed, but the sherds were not burned. All levels associated with House Platform 2 had a preponderance of Mayapan types.

Approximately 9.5 m due east of House Platform 2 a small altar was investigated; directly west of it a cache of five Mayapan-type vessels was found buried 20–70 cm below the surface. The vessels consisted of a large porous gray jar, a red tripod dish with effigy feet (human head with turned-up nose), a small red jar, a red cylindrical tripod vase, and a complicated and badly damaged effigy vessel. Nothing remained of the altar save a large rectangular stone set on edge, facing which was a rectangular paved area. The cache was just west of this paving.

Originally Platform C may have supported three single-room shrines consisting of two nearly square structures opening north and south, respectively, and flanking a larger rectangular construction that faced Cenote Santa Cruz to the west. There is now no trace of a shrine on the south side, but there is ample space on the platform

for it. Both existing shrines originally had beam-and-mortar roofs and a centrally located altar or dais. The pottery collected in the two trenches which cut through the doorways of these shrines and descended to bedrock was predominantly of Mayapan Period with little change from top to bottom. On the other hand, the ceramic pattern indicated in the three levels excavated in near-by Cenote Santa Cruz was one of increase in early types at the expense of late types as the pit progressed downward.

From the ceramic evidence it would appear that Platforms A and B were originally constructed during the Puuc Period and that both were subjected to later building activity. Platform C suggested only late, Mayapan Period construction.

The next work undertaken was at the large mound in the village of Telchaquillo. This ruin, situated south of the cenote in the Main Plaza and still standing after all the years of stone robbing for village property walls and house foundations, remains an impressive mass of masonry. The principal aim in trenching into this large complex was to determine something of its age through its growth, both horizontal and vertical. It was by reason of the horizontal accretions with their time-sensitive pottery inclusions that a chronological sequence developed. That this sequence did not evolve into something more conclusive is probably due to the fact that the mound was not penetrated sufficiently far. The reason for not continuing deeper was the excessive outlay in time and labor that would have been required. The two trenches, one on the south, the other on the west side, each penetrated through several terrace walls. In the pottery lots, Puuc types were always more abundant than Mayapan. Actually, the farther into the mound we progressed, the greater became the predominance of Puuc over Mayapan types. The fact remains, however, that Mayapan wares were always present. The ceramic trend suggests that farther inside the mound a pure sample of Puuc pottery might have been encountered.

## ART AND ARTIFACTS OF MAYAPAN

TATIANA PROSKOURIAKOFF

This season's work has substantially advanced our knowledge of the artistic styles at Mayapan, which document the decay of Maya culture. We were extremely fortunate to uncover a portion of a mural painting on the wall of the front gallery of a large temple, Q-80. The wall is broken by five niches, which, in the composition of the painting, represent doors of buildings depicted around them. The buildings apparently stand on a terrace represented by a black dado at the base of the wall, interrupted, however, by the niches, which extend to floor level. Between the niches on the black dado are depicted heads of monsters with open jaws, which strongly resemble serpent heads carved on the columns of Structure 2C1 at Chichen Itza. These heads are painted in vivid tones of green, red, yellow, and blue, and in white. Above them the mural is largely destroyed, but by combining the scattered features still preserved, one can reconstruct the outlines of the houses that frame the niches.

The upper zone of each house consists of a narrow vertical zone between typical Maya two-member (fillet and bevel) moldings. Below, the walls are not vertical, as in most Maya buildings, but are composed of two sloping zones, the upper one projecting slightly beyond the lower. Some of the buildings at Mayapan have a sloping lower zone, but the upper slope is seen for the first time on this mural in connection with a Maya building. The houses are painted on a red background, and their walls and moldings are brightly decorated with geometric designs in polychrome, largely composed in bands and outlined in black. Around each niche is a T-shaped form framing the lintel and jambs in the manner used in Mexican codices.

The mural extends almost to the vault spring of the gallery, above which there are no traces of plaster to show whether or not the painting was continued on the soffit. Unfortunately the condition of the

plaster and of the masonry behind it is such that it would be very difficult either to remove the painting or to consolidate it in place. It has been carefully reburied in earth and masonry debris, which will keep it better preserved than could any possible attempt at restoration.

The sculptural style of Mayapan, so little known because of the poor preservation of monuments on the surface, is slowly beginning to emerge from a number of smaller carvings coming out of excavations. A grotesque figure, examples of which range between 15 and 45 cm in maximum dimension, appears to be a type of ceremonial object associated with shrines of houses and colonnaded halls and in some way to be related to household worship. Two of these carvings representing turtles have a hollow receptacle on the back. The turtle motif strongly prevails and is usually accompanied by anthropomorphic features. In some cases, the turtle has a human head; in others, a human head is held in the mouth. There are also crouching human figures, some with a mantle on the back strongly suggestive of a carapace. One sculpture, recovered from a house (Structure R-88), seems to represent an armadillo with a human figure on its back. On the sides of the carapace are carved the glyphs 4 Ahau and 13 Ahau, and on the back of the human figure is 1 Ahau. A much smaller carving of a turtle is made of fine polished green stone and is executed with admirable simplicity.

Most of the artifacts recovered this year fall into classes found in previous seasons, but a small fragment of ancient cord and cloth adhering to some copper bells, and preserved by the action of the disintegrating metal, is an unexpected addition to the list. Also new are two fragments of green obsidian, which is rare in Maya collections. The finds of jade continue to be meager and of poor quality. Jade is used mostly in the form of beads, often marred by fire, though there are several well polished jade disks 3-4 cm in diameter, perforated in the center. Shell beads



occur in greater numbers, many in the form of perforated tinklers. Teeth, antlers, sting-ray spines, and bones are also represented, and several more greenstone celts have been found.

Chipped flint implements have produced no strikingly new forms, but the number of arrowheads is increasing both in flint and in obsidian, with the "expanding stem" formed by side notches prevailing as the common form. Also increasing is the incidence of small obsidian scrapers and other tools made by retouching flake-blades.

Limestone tools and occasional tools of harder volcanic and granitic stones continue to come in, but the use of roughly chipped limestone disks about 10 cm in diameter remains a puzzle. They have sometimes been designated "pot lids" but with very little justification.

The items listed in this year's catalogue amount to 294, making a total of 761 for the Mayapan project so far. Since shell beads, copper bells, and other small objects are often listed in groups, the actual number of individual specimens is much greater, even if we exclude the sizable collections of obsidian flake-blades and of flint chips, which are not listed but are being held for future study.

#### CHICHEN ITZA, DZIBIAC, AND BALAM CANCHE, YUCATAN

K. RUPPERT, E. M. SHOOK, A. L. SMITH, AND  
R. E. SMITH

Ruppert and A. L. Smith spent 11 days at the ruins of Chichen Itza for the purpose of exploring the site for house types. They found the task difficult because of the thickness of the vegetation and the poor condition of the structures. A large part of the mapped area was covered, as well as some terrain slightly off the map, and 43 houses were found in good enough condition to yield plans. These were found on low, flat ground and on large platforms, many of which border the sacbes, in Squares 5B, 6B, 7B, 3C, 4C, 5C, 4D, 5D, and 4E, and off the map east of 4E. During

this search for house types two unreported mercado-type structures were discovered.

The house types, although in some cases slightly different, are closely similar to those found at the Puuc sites of Uxmal, Kabah, and Sayil, and are unlike those at Mayapan. Of the 43 houses investigated, 9 were tested for pottery. Four had post-Toltec-Chichen Period pottery above floors, including large effigy censers similar to those found at Mayapan, numerous slipped Red ware jar sherds, many from one vessel, which were not the same as Mayapan Red, and a few black-on-cream sherds. The rest of the pottery from house types, including some from below floors, belonged to the Toltec-Chichen Period. This suggests a late re-use of certain houses.

Shook and R. E. Smith made a ceramic survey of Chichen Itza from March 14 to April 8, during which time a day was devoted to excavations at each of two near-by sites, Dzibiac and Balam Canche. Our objective in making a study of Chichen Itza pottery was to check the range of the site's occupation in the light of established ceramic chronology at Mayapan. In particular, we hoped to encounter refuse deposits of long duration or in association with the well known Maya and Toltec styles of architecture in Chichen Itza. Another objective we believed to be of prime importance for Yucatan and Mesoamerican prehistory was a knowledge of the full range and context of ceramics pertaining to the epoch of Toltec influence in Chichen Itza.

We began the study by making a surface reconnaissance covering the major portion of the area shown on the map of Chichen Itza. It was observed that the map shows the larger structures of the site but fails to include the majority of the lesser mounds. In some groups the latter outnumber the more impressive buildings. Many of the unmapped mounds are ancient houses. They often occur on the same terrace in close association with the larger, and frequently vaulted, structures; others may be on extensive stone-filled platforms

with no large accompanying units. The map shows the platforms, though rarely the low mounds upon them. Large sections of terrain were almost barren of surface pottery or other indications of a concentrated population in ancient times. These conditions prevailed especially in the south-central and southeastern sectors, and in the area lying north and northeast of the great terrace or platform supporting the Castillo, Great Ball Court, Court of the Thousand Columns, and other structures. The preliminary reconnaissance served to identify these zones as unfavorable for the location of deep refuse deposits and to suggest that the best opportunities lay in the immediate vicinity of terraces or platforms on which large vaulted buildings were concentrated. Invariably, the deepest middens were found off the edges of these terraces. The contents of the middens, discussed below, we believe suggests that they were an accumulation in part of household refuse, in part of ceremonial material. That many people lived in close proximity to the large civic and religious buildings seems certain from the evidence.

We made nineteen ceramic cuts at Chichen Itza. The locations of these include the principal groups of the site from its southwest to its northern limits. The procedure, after finding by surface observation a location which appeared favorable for pottery dumps, was to dig a test pit or trench from the surface to bedrock, removing arbitrary levels of 25 cm unless floors or other divisions were encountered. Some of the tests proved unproductive; others cut through stratified refuse. In the latter instance we often enlarged the area excavated to obtain an adequate sample of material.

The pottery from only nine cuts has thus far been recorded and subjected to preliminary analysis. The results are most instructive, in spite of the fact that four of these cuts were ceramically unproductive, lacking stratigraphy and sufficient sherds to do more than date the platform or construction penetrated. Cut 2 was a

good example of a productive cut. There were three strata and a floor sealing off the two lower levels. The latter contained nothing later than Puuc types, whereas the above-floor level yielded mostly Toltec-Chichen types and a few Puuc specimens. Cut 8, with three arbitrary strata, showed a slight change from bottom to top, but all the pottery was within the Toltec-Chichen Period. This same ceramic pattern was found in the four strata of Cut 14, where Fine Orange X formed less than 1 per cent in the two upper strata as compared with 5.3 and 4.9 per cent, respectively, in Strata 4 and 5 of Cut 17, unquestionably the most important cut for establishing Toltec-Chichen ceramic types. These types were present in all six strata of this deep midden. Puuc specimens associated with the three lowest levels were lacking in the upper levels; Chichen Medium Slate ware became more abundant as the surface was approached. Cut 13 was the only one which appeared to harbor post-Toltec-Chichen ceramic types, including unslipped lightly striated jars and black-on-cream vessels similar to those found in the lower levels of the principal period of occupation at Mayapan. These types occurred both below (21 per cent) and above (48 per cent) a well preserved floor. The only other types recognized in this cut were of the Toltec-Chichen Period.

The principal ceramic types of the Toltec-Chichen Period as found in Cut 17 comprised: unslipped jars often with pattern-striated bodies and bolster rims; censers, hourglass type sometimes embellished with buttons or spikes set in a painted blue background, jar-shaped with three hollow bulbous feet, or ladle type, a few of which may have been painted red with a blue lip border; Chichen Medium Slate vessels (jars, bowls, usually without feet save for grater type; restricted-orifice bowls with bolster rim, and rarely bolster-rim basins), which had a light translucent cream slip showing the brick-red paste underneath; Chichen Red vessels (jars, round-sided bowls with flat or impressed



disk base and occasionally with black design; flaring-sided bowls with everted rim and sometimes gouged-and-incised through a cream band; and rarely cylindrical vases), which were somewhat mottled but otherwise smooth and waxy to the touch and quite like the Puuc Red types; Fine Orange X, and a polychrome Fine Orange like that from Isla de Sacrificios, Veracruz; hard yellow ware with black trickle (jars, restricted-orifice bowls with bolster rim, and flaring-sided bowls); and a very few Tohil Plumbate vessels.

The primary facts gleaned from our work are: (1) there is no pottery at this site earlier than the Puuc Period, save for a collection of Formative types from a single cut east of Structure 3C6; (2) there is no pottery later than the Toltec-Chichen Period but for a small quantity of black-on-cream (Brainerd's coarse slate) sherds from Cut 13, a pit just south of the Mercado; (3) the content of the Toltec-Chichen ceramic phase is established by means of Strata 4 and 5 of Cut 17, where the greatest concentration of Fine Orange X was found.

The reason for finding almost no material later than the Toltec-Chichen Period in the nineteen cuts is presumably that the permanent occupation of Chichen Itza terminated at or near the end of that period. The few black-on-cream ware sherds, usually thought to belong to a later period, may have been precursors of, and thus slightly earlier than, the similar, early Mayapan Period type. The explanation for the late sherds found above the floors of certain house-type constructions is probably that people were re-using the houses in late times. These people may have been pilgrims who each year occupied the better-preserved houses during their visits, leaving behind some broken utilitarian pottery as well as censers. That people returned to an abandoned and ruined Chichen Itza to worship is made clear by E. H. Morris (1931, C.I.W. Pub. 406, pp. 179-180) in describing the pottery, mostly of the large effigy-censer variety but including some

red ware tripod plates found "in the vegetable mold just beneath the surface, most plentiful in front of the doorways and strewn down the stairs." He also describes pottery, found on the floors of buildings, which closely resembles what has been listed above under Toltec-Chichen types. These wares were "in use while the buildings were still standing." The findings resulting from the nineteen cuts made by Shook and Smith entirely agree with the conditions described by Morris.

The ruins of Dzibiac were visited briefly. They are situated about 10 km west of Chichen Itza, around a beautiful cenote bearing the same name. We noted several platforms with fallen structures on them and two buildings with standing or partly standing vaulted rooms. The larger of the two lies about 50 m southwest, the smaller some 200 m north, of the cenote. Each has remains of vaulted chambers around two or more sides of a thick masonry core, resembling in this respect many of the palace-type structures of the Puuc region. At Dzibiac the masonry of the room walls consists of roughly cut blocks laid in irregular courses with a considerable number of spalls between stones. The undressed blocks are well tenoned into the hearting, in contrast to the shallowly tenoned veneer stones of Puuc and Toltec-Chichen architecture.

The vault masonry is strikingly different from that used in the walls. The vault stones have semi-specialized, long tenons and are excellently cut and dressed on the exposed faces. They are laid evenly, producing a rather true plane from the offset at the vault spring to another offset just under the capstone. The structure nearest the cenote has a series of small subterranean vaulted chambers honeycombing the central block of masonry. Their size and position suggest that they served as burial chambers, but the local people have a more colorful interpretation. They believe the tiny vaults were the home of elves, the little people, and refer to the ruins by the name *alux*, or dwarf.

We observed no carved stone elements of architectural ornamentation or doorway columns at Dzibiac. The masonry and style of architecture conform most closely to the pre-Toltec buildings in near-by Chichen Itza. The pottery from a trench dug off the west terrace of the building with the burial chambers belongs to the Puuc, or Late Classic, Period.

Shook made a preliminary exploration of the site and cave of Balam Canche, and later returned with R. E. Smith for a more thorough investigation. The ruins surrounding the mouth of the cave have not been reported previously, although the cave has long been known. The site lies 4.5 km east of Chichen Itza and is readily accessible from the recently paved Merida-Valladolid highway. The ruins, only 300 m north of the highway at Kilometer 124.5, served as a stone quarry during the road-building operations. Much of the site was destroyed by this work. Left were remnants of a masonry wall enclosing the cave entrance, a mound, and two vaulted buildings and several low house foundations on what originally had been an extensive platform. The last enclosed the eastern half of a plaza in the center of which is located the cave entrance. The vaulted structures had walls of roughly cut, undressed blocks; doorway jambs of carefully cut and dressed, full-width stones; plain stone lintels; and semi-specialized, well cut and dressed vault stones. None of the building stone observed bore carving. Architecturally, the two vaulted structures in Balam Canche are similar to those at Dzibiac. A surface collection of pottery and material from a ceramic trench through the platform east of the cave indicate a long period of occupation. The major building activities, however, took place during the Puuc, or Late Classic, Period.

The natural cave with its several water sources has been investigated by A. S. Pearse (1938, C.I.W. Pub. 491) and others principally interested in the fauna of Yucatan caves. From time to time selected

samples of pottery were collected from the cave floor. These sherds, studied by Vailant, Roberts, and Brainerd, led to the establishment of a ceramic type which Brainerd calls Incised or Flaky Dichrome and places stylistically in the beginning stage of the Early Classic Period. We made three excavations in the cave floor by the water pools nearest the entrance and collected a large sample of pottery from the surface of the floor throughout the cave. A small stone sculpture depicting a human figure in kneeling position also came from here.

The pottery within the cave, like that recovered from the ruins, showed a long range of occupation. Most abundant on the surface of the cave floor was Puuc Medium Paste Slate ware of the Late Classic Period, and there were a few sherds of the Toltec-Chichen, Mayapan, and Post-conquest Periods. In two excavations, only pre-Classic and Early Classic types were found. The oldest material consisted principally of lustrously polished, red-slipped jars. These do not bear handles of any kind, nor do the Incised Dichrome jars which directly overlie the pre-Classic Red and Orange monochromes. Several sherds cannot be distinguished macroscopically from the Pattern Burnished Ware which Brainerd discovered in the Mani cenote and considers to be the most ancient pottery so far recognized in Yucatan.

#### EXPLORATION IN QUINTANA ROO

G. STRÖMSVIK, H. E. D. POLLOCK, AND H. BERLIN

The explorations described below were carried on by Strömsvik and Pollock between February 23 and 28, and by Berlin and Strömsvik between May 24 and 29, 1954. Previously, one of our workmen from the village of Telchaquillo, Yucatan, had been sent into the area to gather information on the location of ruins and to report on roads and living conditions. His report assured us that we could make the trip by truck and that we could obtain some food supplies and camp facilities at



the village of Ichmul, the site of certain ruins we wished to observe.

The first trip started from Telchaquillo, going by way of Tekit, Teabo, Ticul, and thence to Peto over the newly built highway from Merida to the last-named town. The second trip left from Merida, using the highway to Peto. Beyond Peto, and the end of road normally considered passable by automobile, our course was generally east through the village of Progreso (12 km from Peto), Dzonotchel (24 km), and Calotmul (31 km) to Ichmul (43 km). This is the old road from Peto to Santa Cruz de Bravo, now known as Carrillo Puerto. Immediately east of Calotmul, we stopped to observe a small ruin site of four mounds. Two of the mounds are each about 8 m in height, one being a pyramidal structure, the other probably having been a two- or three-storied palace-type building. On both structures Puuc-type stones are lying about, and part of a vault of very crude masonry is visible near the bottom of the second. The vault cannot be placed as to type in this relatively unknown area. Possibly it is to be associated with the crude vaulting, seemingly of late period, seen subsequently at Okop. The remaining two mounds at Calotmul were smaller and without notable features showing.

Ichmul, which lies just over the border in Quintana Roo, is an old settlement, as is shown by Ciudad Real's mention of the town in the sixteenth century. At present it is more notable for its colonial than for its aboriginal ruins. Overgrown streets and tumbled-down remains of colonial buildings extend a kilometer or more in all directions from the large central plaza. Two imposing churches—the second and bigger one never completed—a cuartel, and an unfinished cabildo face on the plaza. The more important aboriginal remains, consisting of three large pyramidal mounds and the ruins of an enclosed quadrangle, all rising from a huge terraced platform, lie just south of the plaza. Other terraces, courts, and smaller mounds lie farther south, and another group of ruins

was observed east of the plaza of the town. On each of the principal pyramids are elaborate fortifications in the form of head-high walls of stone, clearly of colonial or later origin. Tradition in the settlement, which consists of a schoolmaster and possibly a dozen families that seem only recently to have taken up residence in the abandoned town, is that the fortifications were built at the time of the War of the Castes. The eighteenth-century date of the unfinished church, however, suggests that the place was abandoned at an earlier time.

As might be expected in a town of the size that Ichmul once was, the ancient Maya structures are in a shocking state of ruin. They were undoubtedly used as quarries to build the impressive colonial structures, and the fortifications on each pyramid have leveled the remains of any buildings that may have stood there. In the eastern and largest of the pyramids, about halfway up the mound, we observed and explored a system of vaulted passages that ran into the center of the structure. These were of crude construction, built entirely of roughly shaped but otherwise unworked stone. Toward the western edge of the ruins, just south of the colonial cuartel, the section of a partly fallen vault protruded from the side of a large platform. This was of interest in showing a sequence of different kinds of masonry. The primary vault, which was of Puuc-type construction, was built against, and was clearly later than, a battered wall, apparently the old face of a terrace, of well cut and dressed veneer masonry. This wall might be of Puuc Period or earlier. Inside the vault, and secondary to it, was a smaller vault of crude masonry comparable to that seen in the eastern pyramid. This sequence suggests that the crude style of masonry is later than typical Puuc construction, but we should not put much weight on this single example.

We obtained a small, and hardly adequate, sample of pottery from a single trench and from digging around the western vault in Ichmul. The sherds were

mostly of colonial and Puuc types. There was a sprinkling of sherds similar to southern Classic types. Owing to the difficulty of distinguishing Mayapan Red ware from colonial pottery, in the absence of distinctive rim forms, one cannot be certain that Mayapan Period ceramics are not represented at Ichmul. Only further excavation can answer this question. On the basis of pottery findings and of architecture, one guesses the major period of the site to be Puuc, or Late Classic.

About 4 km south of Ichmul is the abandoned colonial settlement of Querul or Xkerul, a name one suspects to be a Spanish corruption of a Maya word. Halfway along the trail to this site we noted an ancient road, or sacbe, which we followed to its termination at a pyramidal mound north of the ruins of a colonial church. The mound, which stood about 9 m high, was a tumbled mass of fallen stone with no standing masonry showing. It was the only ancient structure, with the exception of the sacbe, seen at Querul. The sacbe is 10–12 m wide and at places stands at least 2 m high. We did not trace the road to its terminus at Ichmul, but there is little doubt that it runs to that site. There is no reason to think that the pyramid at Querul and the sacbe are not of the same period as the ruins of Ichmul.

Strömsvik and Pollock made a trip of a single day to the ruins of Okop, or La Aguada, as the site is locally known, and the subsequent journey of Berlin and Strömsvik was devoted entirely to that site. The ruins lie southeast of Ichmul on the road to Santa Cruz de Bravo. The only sizable settlement passed was Sabán, an old colonial town that appears also to have been abandoned and reoccupied, 23 km from Ichmul. Eleven km farther along one passes a tiny cluster of huts that preserves the name Okop, and 2 km beyond is the small fresh-water lake that gives the name La Aguada to the ruins. There is a group of a dozen mounds 100–200 m west of the lake and another group about 1 km

north. The two are connected by a sacbe 12 m wide.

During the few hours spent by Strömsvik and Pollock at the site, only the northern group was examined, although the existence of the southern was noted. The group consists of three good-sized pyramids, ranging from about 9 m to 15 m in height, and several smaller mounds that rest on a large platform some 150 m north and south, and that much or more in an east-west direction. The pyramids are arranged about a plaza on this elevation. To the east of the pyramids are more mounds, but we did not explore that area. South of the great platform is a small pyramidal mound on the east edge of what seems to have been another plaza or court. In this second plaza, Strömsvik discovered a sculptured stela, half-buried and caught in the roots of a huge breadnut tree. In attempting to extract the stone, we broke our only axe, and it was not until the second trip to the site that the stela was finally brought to full view.

Two of the pyramids, the northern and eastern, support partially standing temple buildings at their summits. These are small, single-room structures built of crude masonry with rough vaults that step inward from the four sides of each room. The northern building has a single column in its doorway. Mixed with the crudely shaped stones of the walls are occasional well cut stones of Puuc type. Over each entrance, in front of the lintel, is a sunken panel, a feature typical of late, East Coast architecture.

The stela, the only sculptured monument discovered at the site, shows a human figure carved in low relief with a short panel of hieroglyphs to the figure's right and a full-length panel to the left. The top of the stela, carrying the head and neck of the figure and the upper parts of the glyphic panels, is broken off and was not found. The glyphs are too eroded to be legible, but a fair amount of detail of the human figure remains. It shows a personage with toes pointed straight out at an



angle of 180 degrees. Across his chest, from lower right to upper left, he clasps a Ceremonial Bar. On stylistic grounds, Proskouriakoff believes that the sculpture can be dated as early in the Late Classic Period, possibly around 9.9.0.0-9.11.0.0 in the Maya calendar.

As previously mentioned, the southern group lies about a kilometer distant from the ruins just described and is connected to them by a *sacbe*. About 50 m short of the southern end of the ancient road, where it merges with a high terrace, is a round stone altar, 1.50 m in diameter, without carving. The principal structure of the group is a pyramid that rises some 20 m above the terrace and possibly 30 or more m above natural ground level. The pyramid faces north; at the foot of the stairway is another circular altar, 2 m in diameter, also without carving. On top of the pyramid are the remains of a small single-room temple building. The strikingly heavy walls which enclose the tiny room are built of the same crude masonry seen in the temples of the northern group, and here again is seen at least one Puuc-type building stone among the otherwise undressed stones.

No other standing masonry was noted here. At least a dozen more mounds make up the group, the most notable being a large enclosed quadrangle just south of the pyramid.

No pottery that might assist in dating the ruins was recovered at Okop. The character of the remains, particularly the stela, suggests that the major period of construction and of occupation of the site was Classic, probably Late Classic. The small temples on top of the pyramids seem to be of East Coast style and presumably are of later date.

#### CERAMIC STRATIGRAPHY OF THE EAST COAST OF YUCATAN

WILLIAM T. SANDERS

Under a Carnegie Institution fellowship, Sanders began an archaeological recon-

naissance of the East Coast of Yucatan. This survey was particularly directed toward learning the ceramic stratigraphy of that area and relating these findings to the better-known pottery of central and western Yucatan. Ever since the publication of Lothrop's monumental work on East Coast architecture it has been known that Toltec (Tula) influence in basic architectural forms, if not appearing in specific details, was general and widespread on the East Coast. The exact relation of this architectural style to that of the Toltec-Chichen and Mayapan Periods in central Yucatan is of great importance to an understanding of late-period history in the northern Maya area. It was hoped that surface collecting of sherds and small-scale excavation at a number of East Coast sites might at least define more sharply, if not solve, those problems. The northern and northeastern coastal areas were selected as the beginning point for this ceramic survey. Most of the coast from Holbox on the north to Tulum on the south was visited and worked. A number of sites in northern inland Quintana Roo were also tested.

One of the principal difficulties in doing work in this area is the lack of transportation facilities and of manpower for even small-scale excavation. It was decided to hire a small crew of workers from the Yucatan-Quintana Roo border town of Kantunilkin for the entire field season. The quality of labor there is unusually high. The season was started at the often noted but never adequately described site, which is of some size, situated in the village of Kantunilkin. It is an excellent jumping-off point for field work in northern Quintana Roo, there being two routes open to the coast. One is passable with jeep or truck and runs almost due north to the village of Solfarino about 30 km away. From Solfarino there is a typical Yucatecan *tranvia*, or narrow-gauge rail line, which goes as far as Chiquila on the coast, just opposite Holbox and at a distance of about 15 km. Along this route and the shores of

Laguna de Yalahau six sites were visited and excavated.

The second route is a mule trail which goes from Kantunilkin to Leona Vicario (Santa Maria), about 30 km. From Leona Vicario there is another tranvia, about 40 km in length, which goes to the coast, terminating at Puerto Morelos. Four inland sites were excavated along this tranvia.

Most of the remainder of the season was spent visiting and testing sites on the mainland, all within a kilometer of the beach, from El Meco, just opposite Isla Mujeres, in the north, to Tulum in the south. Three weeks were spent on Cozumel Island, an area rich in late-period sites. Fifteen sites were tested on the mainland, including such well known centers as Tulum, Tancah, Xolha, Palmul, and El Meco. Approximately eleven sites were excavated on Cozumel Island. Most of these places were reached by boat; actually, more mileage was covered on foot, during the season as a whole, than by any other mode of travel.

At some sites adequate sherd samples were impossible to obtain because of the rocky terrain and the exceeding smallness of the site, in certain cases a single shrine situated on a rocky beach head. In at least twenty sites, however, good to fair sherd samples were obtained, although good building or occupation stratigraphy was rarely met.

Besides being tested for ceramic data, a high percentage of the sites were at least roughly mapped, and standing structures were photographed and drawn. Extensive trail cutting was done in the vicinity of ceremonial centers to obtain data on house-mound types and their density. Also, information was collected on agricultural techniques and production by interrogation of workers from Kantunilkin, Leona Vicario, and Cozumel.

In general, the sites found or revisited are small and not very impressive in size, in quality of architecture, or in number of sculptured monuments. The largest seen was Tulum. The average site in the area is

of one of two general types: (1) a single-plaza ceremonial center surrounded by from three to five buildings, or (2) an isolated single-temple, or at times two-temple, beach shrine. The East Coast architectural style as defined by Lothrop seems to be a strictly coastal phenomenon, not seen at any of the inland sites visited.

Chronologically, the sites excavated run from Early Classic to the conquest. This dating is based on architectural notes and a preliminary look at the pottery. Red ware is abundant, and some of it looks like Mayapan Red. Censer wares certainly related to Mayapan varieties are also common. Fine Orange is present at a few sites, but is exceedingly rare; no plumbate was noted.

#### TABASCO AND CAMPECHE

HEINRICH BERLIN

As the surface collections of the previous field season in Tabasco had revealed a great abundance of fine paste wares (including Fine Orange), the 1954 season, again under a Carnegie Institution fellowship, was devoted to carrying out excavations at six sites representing Late Classic and post-Classic horizons, in order to obtain larger and stratigraphic samples of this important material. Berlin was in the field from January 11 to April 15. Until February 27 he was accompanied by Carlos Navarrete, a student of the Escuela Nacional de Antropología e Historia of Mexico City, with whose assistance it was possible to run a greater number of test pits.

At the beginning of the season the ruins of Palenque were visited. The director of the local school at Palenque village possessed a painted stucco glyph which he had found at the temple of the near-by site of Xupa. Thus it seems likely that the piers of this small temple had stucco inscriptions in the fashion of the Palenque temples, a fact which had escaped all previous visitors. A careful search at the foot of each pier should result in rewarding finds.

The first dig was done at Tecolpan, be-



low Emiliano Zapata, on the left bank of the Usumacinta River. Pits were opened at several places near the base of the main pyramid, in the surrounding swamps, and on an elevation in the swamps. Only a few surface sherds were found in the swamp pits, probably coming from backwash from the main group. This absence of sherds in the swamps is evidence that in prehistoric times this region already was swampy and therefore not inhabited. On the other hand, in the pit on the elevation, probably a house mound, sherds were found almost to the ground-water level. The digging around the main pyramid duplicated the findings of last year. Several burials were encountered in the various test pits, but none was associated with any ceremonial offering, nor was there any consistency in their orientation, though supine position was predominant.

At near-by El Tren a few pre-Classic and Early Classic sherds had been picked up last year. This season, unfortunately, only one day could be devoted to the making of a hasty test pit. It yielded a high percentage of Early Classic material, showing in the polychrome ware notable similarities in design and color to the Peten material of the same epoch. Yet in the Late Classic pottery of Tecolpan, Tepeu-like polychrome is virtually absent.

The Late Classic fine paste horizon was met again at Jonuta. The character of the deposit and stratigraphic situation there are peculiar: a black, 1-m-thick layer, heavily packed with sherds, emerges from the Usumacinta waters just northeast of the town and, after gradually rising for 30 m, reaches the top of the right bank, continuing southwest for several hundred meters. It is in this layer that the famous Jonuta figurines occur. A fine collection of figurine heads—mostly belonging to whistles—in the possession of Sr. Omar Huerta was photographed. Despite the great variety, recurring types could be recognized and classified into major groups. In a subsequent visit to the local museum of Villahermosa, several Jonuta subtypes were

identified in the figurine collection on exhibit.

At this museum are housed all monuments of the Tortuguero ruins, brought there since last year. They can now conveniently be studied. In addition to the monuments already known, two hitherto unknown fragments were brought to the museum. These are Monument 7, the trunk of a statue of which only the back is in fair condition; and Monument 8, probably the lower part of a standing sculpture. The first is similar to Monument 3.

The sherds excavated at Huimango in the Cunduacan district seem to belong to the Late Classic Jonuta horizon. Fine paste ware at Huimango, however, is more of a gray variety than of the accustomed orange.

Another site where excavations were undertaken was Juarez in the Cintla region, close to the battlefield where Cortez won his famous victory over the Indians of Tabasco. The top of the main mound was cleared and much ceremonial ware recovered, such as fragments of incense burners, ladle incense burners, small atlantean figurines, and clay models of temples. Human faces and general workmanship are extremely crude. The associated fine paste wares show almost no decoration and are radically different from the ones found at Tecolpan and Jonuta. All the material was concentrated on the rear of the top platform of the mound and was covered only with an insignificant layer of humus. Evidently it represented the last offerings deposited there before the site was abandoned as a religious center. As no more recent material has been found so far in this region, we can hardly be wrong if we interpret the Juarez pottery as belonging to the last few centuries prior to the conquest.

A similar situation was met at Tamulte de las Sabanas. On the rear of the top platform of the main mound of a major group, ceremonial and fine paste wares again were encountered, in this case associated with copper bells, a definite proof of relatively recent age.

In the modern village of Tamulte there still exists a local tradition of pottery making, done by women only. This pottery is not traded outside the village. Besides the normal ollas, comales, and so on, they make potstands (called *met* in the native Chontal language) and heavy-walled tubes (*xun*), which are used three at a time as firestones, a suitable substitute in a region where stone is completely lacking.

Finally, at Atasta (Campeche) a very low platform was excavated and a small earth mound trenched. On the platform was a modeled stucco jaguar which had formed part of its decoration. The façade of the mound was built only of stucco without the use of any stone. At the center two legs of a person in dancing attitude were still recognizable. In front of it was a small round altar platform, the walls of which were also done in stucco.

The trenching of the mound resulted in the discovery of two minor graves, one at the level of its foundation, the other near the top of the mound. The ceramic material recovered from both graves belongs to the same epoch, the previously established post-Classic fine paste coastal horizon. One should bear in mind, however, that the excavations at Atasta were limited to a few acres and that the real history of the site and its immediate neighborhood probably extends over a much longer period of time.

The excavations of 1954 in Tabasco have revealed two fine paste ware horizons. One is Late Classic and centers around Jonuta, where it is associated with beautifully executed Maya figurines. The other is post-Classic or even Protohistoric and is found along the Tabasco and southwest Campeche coast. One is tempted to ascribe this last material to the Chontal-speaking tribes which inhabited the Tabasco coast at the time of the conquest and still inhabit part of it. The change in the aesthetic concepts may be due to the influence of foreign groups producing a stimulus which the coming of the Spaniards prevented from developing a fresh art style.

## CERAMIC TECHNOLOGY

ANNA O. SHEPARD

Ceramic technological work has been directed mainly to studies of general application. This course has seemed advisable because of the delays and difficulties involved in bringing adequate samples of Yucatecan material to this country. A number of problems were suggested in the course of the final revision of Shepard's forthcoming monograph, *Ceramics for the Archaeologist*. These can best be understood in the light of the general status of ceramic studies in American archaeology today. Most studies are based on, or center about, the concept of the pottery type. The pottery type serves as the tool of classification and is an important means of arriving at general chronological ordering of archaeological material. It is usually defined empirically, that is, by sorting potsherds according to the likenesses and differences that can be recognized by unaided visual inspection. There has been much discussion in recent years of the meaning of the pottery type, whether it represents a segment from a continuous course of gradual change, the limits of the segment being artificially determined by the archaeologist's interest and purpose and the points in time and space at which he samples, or whether the type has a more direct basis in the nature of pottery itself, and is at least a node in the stream of a people's ceramic history. Despite differences of opinion on this question, there is general agreement that the pottery type has been an extremely useful means of establishing frames of relative chronological and spatial reference. At the same time, there is a growing feeling that more thought should be given to the filling of these frames.

When cultural content is brought down to the ceramic level, the pottery type is not an entirely adequate tool with which to work, largely because it is defined in the main from sherds and in terms of field criteria. Consequently, the aspects of design and shape style that are considered are



necessarily simple and limited because of dependence on fragmentary material. Since criteria are determined by ease of recognition without laboratory aid, the potter's techniques are considered only in so far as they produce some conspicuous effect, and function rarely enters the picture, since the important uses of vessels are widely shared by peoples and are persistent in time. When pottery is studied as a cultural object rather than as a means of tagging relative periods of time, the subjects of style, technique, and function must be approached directly. Although such studies are independent of established taxonomic systems in method as well as objective, they nevertheless contribute to the methods of classification used in chronological ordering. The distinctive features of design and shape that can be defined from the analysis of large collections of entire vessels extend and sharpen the criteria applicable in sherd classification, since a fragment of a known whole can be recognized, whereas an unknown whole cannot be reconstructed from a fragment. The diagnostic marks of specialized techniques likewise furnish useful and significant means of classification. Criteria of this kind make the pottery type less artificial and give it more value in cultural studies.

The identification of symmetry has offered one means of broadening the study of geometric design styles. Other approaches have been suggested, and some experimental work in perception has been undertaken to test their applicability. Since this work has been largely theoretical, it has seemed advisable to apply the proposed principles to a well known style. For purposes of illustration, Classic Mesa Verde was chosen and analyzed with relation to structure, organization of elements, balance of area and line and of dark and light, spatial relations, and the relation of symmetry to dynamic quality. The practicability of this type of analysis was satisfactorily demonstrated. The significance of the qualities studied lies in the new

basis they afford for investigating the origin and development of styles.

In the field of ceramic technique, firing method is of basic importance. The relation of firing conditions and color has interested the archaeologist for some years, and the example of Southwestern archaeologists in using estimates of firing atmosphere in classification is influencing workers in other fields. There are many pitfalls in judging firing atmosphere from color; for example, incomplete oxidation of a carbonaceous clay, and smoking or smudging are sometimes confused with reduction. The initiation of experiments with firing atmosphere was mentioned in Year Book No. 52 (p. 255). In the past year, this work has been continued, principally by qualitative testing of sherds for carbon and ferrous iron, designed to throw light on the various factors causing gray color and to answer such questions as: Is the color caused by carbon or the lower oxides of iron or a combination of the two? Was carbon an original constituent of the clay (i.e., derived from carbonaceous material) or deposited from smoke during firing? Was ferric oxide reduced in firing, or does the presence of a lower oxide merely indicate incomplete oxidation? The microchemical test used for carbon is sensitive and simple to perform. A small sample of powdered sherd is mixed with an equal amount of copper oxide and introduced into a small bulb blown on the end of a piece of 6-mm glass tubing. The tube is then filled to a depth of a few centimeters with copper oxide, a plug of glass wool is introduced, and above this a capillary is drawn and bent for convenience in introducing it into a milliliter of limewater held in a 2-ml centrifuge tube. When the sample in the bulb is heated gently, the presence of carbon is shown by the formation of a white precipitate of calcium carbonate in the limewater. This is an old standard test adapted to microsamples. Since sherds may contain calcium carbonate which will decompose on heating and give a test for

carbon, it is necessary first to treat the sample with dilute hydrochloric acid and then wash and dry it. Thermal experiments have shown that the carbon deposited in firing is more loosely held and readily oxidized than the hard carbon derived from certain types of carbonaceous matter. A sherd is therefore tested for carbon in its original state and subsequently with samples heated in an electric resistance furnace to successively higher temperatures, beginning at 500° C and taking intervals of 100° C as long as a positive test is obtained. Types of carbon are thus distinguished in sherds whose color gives no clue to differences in firing techniques. Sherds that require a temperature of 700° C to remove all carbon frequently give a positive test for ferrous iron with potassium ferricyanide, and the presence of ferrous iron in the absence of carbon or its persistence after oxidation of carbon is sometimes demonstrated. Grays caused by smoking, lack of oxidation of carbon from carbonaceous matter, and ferrous oxide can thus be distinguished. Proof of reduction in firing requires more than demonstration of the occurrence of ferrous iron oxide, however, since the latter may have originally been present in the clay. Identification of the raw clay and study of effects of firing on other materials, especially iron oxide pigments, is necessary to prove that reduction occurred during firing.

Another project bearing on technique and also on the relation of form and function is a systematic survey of ethnological literature. It is well recognized that the practices of living potters often throw light on prehistoric methods by drawing attention to the marks of various specific methods, the significance of which otherwise escapes notice. To facilitate this review and subsequent comparative studies, two bibliographic files of punched cards have been started: one coded for author, area, tribe, and the full range of significant techniques; and the second, substituting shape and function for technique.

## MAYA AGRICULTURE

JOSEPH A. HESTER, JR.

The 1953 field season, September through December, had a threefold purpose: to supplement a study of Maya agriculture begun in 1952, to compare Maya agriculture with surviving primitive agricultural practices on the Mexican mainland, and to make a cursory examination of archaeological ruins for possible aboriginal units of measure.

From the lowland Maya area in Chiapas, Campeche, Yucatan, Quintana Roo, and British Honduras were gathered data on approximately two dozen varieties of maize which can be considered native to the peninsula of Yucatan. These all, however, are tentatively identified as hybrid derivatives of no more than three of the group. The three basic races of Yucatan maize seem to be *nal tel*, small, yellow-grained, and precocious; *dzit bacal* (*olotillo* on the mainland), a slender-cobbed, eight-rowed white maize; and *xnuc nal* (*tuxpeño* in Veracruz), largest of Yucatan varieties, considered by some to be related to the "Southern Dents" of the United States. Through the courtesy of the Rockefeller Foundation, facilities were made available at the national agricultural college at Chapingo, Mexico, for a study of collections of Yucatan maize.

Certain basic differences between highland and lowland tropical primitive agriculture were noted. These could be attributed, not to apparent cultural differences, but to geographical differences, chiefly of temperature and precipitation. Abundant crude agricultural terracing was observed in highland valleys from the state of Hidalgo to southern Chiapas. Occasionally rock-faced, the terraces for the most part are earth-faced or retained by rows of agave or other plants. Many terraces seem to have been abandoned for a long time, the abandonment often leading to disastrous and irreparable erosion.

In an effort to detect a consistent system of measure, measurements were taken



of basic structural lengths and intervals, and of decorative elements, on ruined buildings at Tula, Teotihuacan, Monte Alban, Zempoala, El Tajin, Palenque, Uxmal, Chichen Itza, and Xunantunich. A special, but inconclusive, effort was made to compare the Temple of Quetzalcoatl at Tula, Hidalgo, with its Yucatan counterpart, the Temple of the Warriors at Chichen Itza. Although a near periodicity was found in the recurrence of certain lengths in the basic design of these two and other structures, no consistent unit of measurement was found to emerge clearly through the crude workmanship. More than a score of contemporary Maya terms for units of measure were recorded from informants in Telchaquillo, Yucatan, and in Soccotz, British Honduras, together with data on the manner in which these units are taken and the purposes for which each is used.

#### PUBLICATIONS

MARGARET W. HARRISON

Volume XI of Contributions to American Anthropology and History (Publication 596) was published in January 1954. Five papers constitute the volume: *Geologic observations on the ancient human footprints near Managua, Nicaragua* (no. 52), by Howel Williams; *Mound E-III-3, Kaminaljuyu, Guatemala* (no. 53), by E. M. Shook and A. V. Kidder; *Conquest sites and the subsequent destruction of Maya architecture in the interior of northern Yucatan* (no. 54), by Ralph L. Roys; *The ball courts at Copan, with notes on courts at La Union, Quirigua, San Pedro Pinula, and Asuncion Mita* (no. 55), by Gustav Strömsvik; and *Pottery from Chipoc, Alta Verapaz, Guatemala* (no. 56), by Robert E. Smith.

The first two papers in volume XII of the Contributions (Publication 606) will appear in July and September, respectively: *The Maya katun prophecies of the Books of Chilam Balam, Series I* (no. 57), by Ralph L. Roys; and *Varieties of classic*

*Veracruz sculpture* (no. 58), by Tatiana Proskouriakoff. These contributions, composed on the IBM typewriter in the Institution's Office of Publications and printed by offset, are being issued as preprints, thus enabling the material to reach readers with a minimum of delay.

Also in early fall will appear *Basket Maker II Sites near Durango, Colorado* (Publication 604), by Earl H. Morris and Robert F. Burgh. This report, printed in the same format as contributions 57 and 58, presents the results of archaeological field work in 1938 and 1940 in the Animas Valley north of Durango, La Platte County, Colorado. Several collaborators recorded in appendices material relative to the archaeological studies: pictographs, by Helen S. Daniels; skeletons, by Charles E. Snow, Professor of Anthropology, University of Kentucky, and William T. Sanders, Carnegie Institution Fellow in the Department of Archaeology; plant materials, by Volney H. Jones and Robert L. Fonner, of the Museum of Anthropology, University of Michigan; animal and bird bones, by Hugo G. Rodeck, Director of the Museum, University of Colorado.

*Bonampak, Chiapas, Mexico* (Publication 602) is still in the hands of the printer.

The manuscript of *Pottery of Uaxactun, Guatemala*, by Robert E. Smith, completed in January 1953, was accepted a year later for publication by the Middle American Research Institute of Tulane University.

A manuscript by A. Ledyard Smith, *Archaeological Reconnaissance in Central Guatemala*, was submitted for publication in the spring. This report covers 68 sites in the highland area; some were rapidly explored, others were excavated in varying degrees of intensity.

From Anna O. Shepard has been received a manuscript, *Ceramics for the Archaeologist*, on which editing has begun.

Three papers opened the fifth volume of Notes on Middle American Archaeology and Ethnology: *Ceremonial or formal*

*archway, Uxmal* (no. 116), by A. L. Smith and Karl Ruppert; *Miscellaneous archaeological specimens from Mesoamerica* (no. 117), by A. V. Kidder; and *Pottery specimens from Guatemala: I* (no. 118), by Robert E. Smith.

The new series of papers issued by the department, Current Reports, inaugurated last year under the editorship of H. E. D. Pollock, concluded its first volume with the publication of eight additional titles: *Archaeological reconnaissance in Tabasco* (no. 7), by Heinrich Berlin; *A portal vault*

*and temple at Mayapan* (no. 8), by Gustav Strömsvik; *Some small ceremonial structures of Mayapan* (no. 9), by Robert M. Adams, Jr.; *Excavations in house mounds at Mayapan: II* (no. 10), by A. L. Smith and Karl Ruppert; *The X-Coton temples at Mayapan* (no. 11), by Edwin M. Shook; *Cenote exploration at Mayapan and Telchaquillo* (no. 12), by Robert E. Smith; *Boundary walls and house lots at Mayapan* (no. 13), by William R. Bullard, Jr.; *Three temples and their associated structures at Mayapan* (no. 14), by Edwin M. Shook.

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